









CHEMISTRY OF  
*Food and Nutrition*



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# CHEMISTRY OF

# *Food and Nutrition*

by HENRY C. SHERMAN, PH.D., SC.D.

*Mitchill Professor of Chemistry, Columbia University*

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*New York*

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## PREFACE

THE PURPOSE of this book is to present the principles of food chemistry and nutrition, with such of the scientific facts as space permits, and as are deemed most important to an effective grasp of the subject. Food is here considered chiefly in its relations to nutrition, inasmuch as the fuller description of individual articles of food and the chemical and legal control of food industries have been treated in another volume.

The present work is the outgrowth of several years' experience in teaching the subject and is published primarily to meet the needs of college classes. It is hoped that the book may also continue to be of service to other readers who are interested in the kinds and amounts of elements and other foodstuffs that are needed in our nutrition, the considerations which should underlie our judgments of food values, and the choice and use of foods for the nutritional improvement of life.

Recent research in the chemistry of nutrition has brought to light many new facts, including several discoveries and chemical identifications of previously unknown vitamins; and also has greatly deepened our insights into fundamental principles. Nutrition has been found to play an important part in the maintenance of the body's reputed "steady states," its relatively constant internal environment. Yet also it is now found that this internal environment is not so rigidly fixed as had been supposed, and is influenced for better or worse by what we take into the body as food, even within the range of conditions universally accepted as normal.

In order thoroughly to incorporate the recent advances, every chapter in this volume has been carefully revised, the latter half of the book has been completely rewritten, and three new chapters, dealing with the most lately discovered among the vitamins, and the relations of food supply to pellagra and its associated deficiency diseases, have been inserted.

All statements of proximate composition and energy values of foods are brought into agreement with the new Government tables. Vitamin values are revised throughout to take account

of all evidence available, and are stated in terms of International units, or weights of the actual substances, or both. With the introduction of rapid *in vitro* methods, quantitative determinations of vitamin C have become sufficiently numerous to permit of a table in Chapter XVII of mean values with their probable errors. In most other cases, however, an attempt to reduce the quantitative evidence on the value of a food as source of a vitamin to a single average figure would at present be premature and would lead to confusion as different averages appeared in different publications. On the other hand, statements of the maximum and minimum of published figures often give greatly exaggerated impressions of variability. Hence most of the vitamin values of foods in this book are given in terms of a reasonable range within which one may expect with considerable confidence that the true average will ultimately be established. The data for mineral elements in foods have been very extensively revised for this edition, and new averages are here given which take account of all evidence available to the end of 1940, including among many others the comprehensive and helpful contributions of McCance and Widdowson. It is gratifying to find that the new data, while extensive, make in most cases relatively little difference in the averages, indicating that in general the averages now given may be regarded as fairly well established. To what extent individual cases vary around these mean results is considered in Chapter XIV and in the Appendix. A new table of copper and manganese contents of foods is included in Appendix C.

*The vitamins are in this edition emancipated from alphabetical order in the interest of a new sequence which with the introduction of the new material seems more logical and teachable; but the general arrangement is otherwise similar to that of the preceding edition.*

Throughout the book, special attention has been given to the interpretive task which seems of outstanding importance at the present time; namely, to show the far-reaching significance of recent discoveries of fact and advances of fundamental concepts in the science of nutrition, while at the same time safeguarding against exaggerated impressions.

With space adjusted to the objective of greatest usefulness of the book to the majority of its readers, a well-balanced account of present knowledge as a whole necessitates a rather strict con-

ciseness of treatment. Hence for the convenience of those who may wish to go farther, generous lists of suggested readings are provided, and where good abstracts are much more generally available these are indicated in addition to the original references. The forms of citation combine the usages of the American Chemical Society, the Carnegie Institution of Washington, and the Journal of Nutrition. In cases of differing usage, the text gives preference to the anglicized forms of proper names as well as of other words.

While the author alone is responsible for any faults of omission or commission, he would gratefully acknowledge the scientific guidance and collaboration of Professors M. L. Caldwell, C. G. King, Grace MacLeod, M. S. Rose, and A. W. Thomas, Doctors H. L. Campbell, C. S. Lanford, and W. B. Sherman, and Miss Anna Zmachinsky.

H. C. S.





# TABLE OF CONTENTS

| CHAPTER  | PAGE |
|--|------|
| I. GENERAL INTRODUCTION . . . . .  | 1    |
| II. CARBOHYDRATES . . . . .  | 10   |
| III. FATS AND LIPOIDS: LIPIDS (LIPINS) . . . . .   | 24   |
| IV. GENERAL CHEMISTRY OF THE PROTEINS AND THEIR AMINO ACIDS . . . . .                                  | 42   |
| V. NUTRITIONAL CHEMISTRY OF THE PROTEINS AND THEIR AMINO ACIDS . . . . .                               | 62   |
| VI. ENZYMES AND DIGESTION . . . . .  | 81   |
| VII. THE FATE OF THE FOODSTUFFS IN METABOLISM . . . . .  | 105  |
| VIII. THE FUEL VALUE OF FOOD AND THE ENERGY REQUIREMENT OF THE BODY . . . . .                          | 123  |
| IX. THE BASAL ENERGY METABOLISM, REGULATION OF BODY TEMPERATURE, AND SPECIFIC DYNAMIC ACTION . . . . . | 150  |
| X. TOTAL ENERGY METABOLISM AND FOOD REQUIREMENT . . . . .  | 180  |
| XI. QUANTITATIVE ASPECTS OF PROTEIN NEEDS AND VALUES . . . . .   | 193  |
| XII. MINERAL ELEMENTS IN FOODS AND NUTRITION . . . . .   | 219  |
| XIII. NUTRITIONAL ASPECTS OF ACID-BASE BALANCE . . . . .   | 238  |
| XIV. QUANTITATIVE ASPECTS OF CALCIUM AND PHOSPHORUS NEEDS AND VALUES . . . . .                         | 251  |
| XV. IRON AND COPPER IN FOOD AND NUTRITION . . . . .  | 278  |
| XVI. IODINE IN NUTRITION: SIMPLE GOITER AS A NUTRITIONAL PROBLEM . . . . .                             | 307  |
| XVII. ASCORBIC ACID (VITAMIN C) . . . . .  | 321  |
| XXVIII. THIAMIN (VITAMIN B <sub>1</sub> ) . . . . .  | 348  |
| XIX. RIBOFLAVIN (FORMERLY CALLED LACTOFLAVIN, FLAVIN FACTOR, OR VITAMIN G) . . . . .                   | 373  |
| XX. NICOTINIC ACID AND THE PELLAGRA PROBLEM . . . . .  | 385  |
| XXI. OTHER WATER-SOLUBLE VITAMINS AND SUBSTANCES OF RELATED INTEREST . . . . .                         | 391  |
| XXII. VITAMIN A AND ITS PRECURSORS . . . . .   | 402  |
| XXIII. THE VITAMINS D . . . . .  | 429  |
| XXIV. OTHER FAT-SOLUBLE VITAMINS . . . . .   | 447  |
| XXV. THE NUTRITIONAL CHEMISTRY OF REPRODUCTION AND LACTATION . . . . .                                 | 456  |
| XXVI. SOME CHEMICAL ASPECTS OF GROWTH AND DEVELOPMENT . . . . .  | 470  |

| CHAPTER   | PAGE |
|---|------|
| XXVII. DIETARY STANDARDS, THE DIFFERING RATIOS OF ADEQUATE<br>TO OPTIMAL INTAKE, AND THE PRINCIPLE OF NATURAL<br>WHOLES . . . . . | 489  |
| XXVIII. CONSCIOUS CHEMICAL CONTROL OF THE INTERNAL EN-<br>VIRONMENT: THE PROBLEM OF THE BEST USE OF FOOD                          | 504  |
| XXIX. FOOD ECONOMICS IN THE LIGHT OF THE NEWER CHEMISTRY<br>OF NUTRITION . . . . .  | 522  |
| XXX. NUTRITIONAL CHEMISTRY AND HUMAN PROGRESS . . .   | 536  |

## APPENDICES

|  |     |
|--|-----|
| A. FACTORS FOR CALORIFIC VALUES OF VARIOUS MATERIALS WHEN<br>BURNED IN OXY-CALORIMETER . . . . . | 553 |
| B. PROXIMATE COMPOSITION AND ENERGY VALUES OF FOODS . .  | 555 |
| C. MINERAL ELEMENTS IN FOODS . . . . .   | 561 |
| D. VITAMIN VALUES OF FOODS . . . . .   | 568 |
| E. SIMPLE STATISTICAL TREATMENT OF THE DATA OF NUTRITION<br>INVESTIGATIONS . . . . .             | 573 |
| INDEX . . . . .  | 579 |

CHEMISTRY OF  
*Food and Nutrition*



# CHEMISTRY OF FOOD AND NUTRITION

## CHAPTER I

### GENERAL INTRODUCTION

"CHEMISTRY is the central science." It links the mathematical with the natural sciences and partakes of the properties of both.

Moreover, it is well to remember that all so-called divisions of science into sciences are only man-made conventions: Nature recognizes no such grounds for separation of scientific subject matter. Particularly there can be no real boundaries between chemistry, physics, and physiology. Each overlaps both of the others.

The interpenetration of chemistry and physics has been a matter of course in both teaching and research for a generation past, and with epoch-making results too familiar to need recounting. More recently has come the realization that chemistry is equally chemistry whether studied *in vitro* or *in vivo* and that the science of chemistry is enormously enriched by this broadening on both its physical and its physiological sides.

This broadening of the chemical point of view also adds greatly to its effectiveness in human service. The chemistry of food and nutrition is now, perhaps, the best developed meeting ground of chemistry *in vitro* and chemistry *in vivo*. Here each broadens the point of view induced by the other.

The objective of scientific research has been well characterized as "to render more intelligible the world in which we live." From this point of view, the two grand divisions of scientific insight are: into the structure of matter; and into the processes of life.

Among modern developments adding to our insight into the processes of life, enabling us to understand these processes better, and in some measure to control and improve them, one of the most

important now appears to be the recent growth of knowledge in the chemistry of food and nutrition.

Thus the subject appeals both to *the spirit of wonder* and to *the spirit of service*. It functions importantly in the two-fold way that Francis Bacon had in mind when he said that science is both for the enlightenment of the mind and for the amelioration of man's estate.

In this subject and in our day, the phrase "amelioration of man's estate" suggests service both to his economic condition on the one hand, and, on the other, to his health and efficiency. Both these, of course, minister to his comfort and happiness: both help us, as Whitehead puts it, "to direct our actions to achieve predetermined ends."

There are also professional reasons for the development of our present subject as a branch of chemistry sufficiently recognized to have its own literature and to be in some measure taught in separate courses.

From the viewpoint of the functioning of science in the world of affairs, it is highly significant that in many countries the food industries far outrank other industries economically. It is estimated, for instance, that in the United States, food products equal or exceed in economic value the total of all other farm products plus all the products of the mines and forests. And chemistry functions largely even in those of the food industries which may not be considered as primarily chemical; for they employ many chemists, and also their products come under chemical inspection and control in the interest of the consumer as they pass into commerce.

From the viewpoint of consumption and cost of living, it is still true for the majority of the world's people, and nearly true for the majority of American families, that, "Half the struggle of life is a struggle for food."

For the more fortunate minority, food does not demand so large a percentage of the income; but at the economic level upon which the majority of families still live, about as much of effort or earnings must be spent upon food as upon all other items in the cost of living if health and efficiency are to be maintained. This fact calls for a large amount of chemical service in the enforcement of laws for the protection of consumers against adulteration and misbranding (or other misrepresentation) of foods. It also calls for the teaching of food values in the schools and colleges and directly

to the public. And it has stimulated widespread chemical research upon the principles of nutrition and upon the nutritive values of foods.

### Major Aspects of This Study

(1) At the turn of the century, the outstanding news in nutrition was that relating to "the man in the copper box," *i.e.*, to the experiments with the respiration calorimeter by means of which the energy relations of nutrition were being established, quantitatively and directly on man, and the foundations thus laid for the building of human nutrition to the pattern of an exact science. These respiration calorimeter experiments were markedly successful. Results of high precision were obtained; and the agreement between the direct measurements of energy (appearing finally in the form of heat) and the computed heat equivalents of the measured chemical exchanges was so close that it became possible (as described in Chapters VIII and IX) to use much simpler and less expensive methods, so-called indirect calorimetry, thus extending the energy aspect of the chemistry of nutrition into the study of a wide variety of problems.

(2) Largely simultaneously with this development of the study of energy relations, but reaching its climax a little later, came a truly epoch-making period of experimental enlightenment in the nutritional chemistry of the proteins and in the protein chemistry of nutrition. The proteins which Osborne in this country, Hopkins in England, Fischer in Germany, and other investigators had studied as chemical individuals and with respect to their amino-acid constitution, were now fed experimentally, first one at a time, then as supplemented by other proteins or by individual amino acids; and thus the chemical natures were correlated with the nutritional functions and relationships. (Chapters IV, V, VII, and XI.) Through such work it has been found that some of the amino acids which the food proteins yield are more or less interchangeable, while some are individually essential — "nutritionally essential" in the sense that they must be supplied by the nutrient (the food protein or proteins). It has also been found that food proteins or their amino acids are the antecedents or precursors of several (at least) of the body's catalysts which are necessary in order that the chemical reactions involved in digestion and nutrition shall run fast enough to support the life process.



(3) Gradually during the past thirty-odd years, in part following upon the protein developments just mentioned and in part independently, there has come an awakening to the importance of some of the mineral elements in nutrition, and correspondingly in the nutritive values of foods. The fact that some of the mineral elements are essential to the anatomical structure and the nutritional functioning of the body has long been known. But for some time the investigations of this aspect of human nutrition consisted of relatively few and rather primitive experiments with diets in which the mineral elements were so drastically reduced that the results appeared rather artificial and failed to bring realization of the truly scientific and practical importance of these elements in normal nutrition and in food values. Within the first decade of the twentieth century, systematic work had been begun to establish the quantitative needs for these elements in nutrition, and their quantitative distribution among the chief articles and types of food (Chapters XII–XVI, XXVII–XXX). Some of these mineral elements are prominent in life processes and important as factors in food values because of very uneven distribution in food and because a food supply chosen freely but without scientific guidance may be in real danger of shortage of one of these elements, even if acceptable to the senses and customs of the consumer and nutritionally adequate in other respects.

(4) Almost simultaneously with the awakening to the significance of the mineral elements in foods and nutrition, and doubtless obscuring it in the minds of many, there has come a dramatically rapid series of important discoveries of the existence in foods and the importance in nutrition of a whole group of substances, called the vitamins. We now know that they are not so related in their chemical natures or in their nutritional functions as to constitute a natural group. Scientifically, the different vitamins should not go together under any group name. That they happen to do so is chiefly the result of two facts: They were all discovered through the growing use of the same general type of innovation in chemical work, namely, the use of laboratory animals as instruments and reagents of chemical research; and they were discovered, through their nutritional behavior, in too rapid a succession for their actual isolation and structural identification to keep pace. Thus the vitamins constitute the most definitely dated but perhaps also the most heterogeneous (Chapters XVII–XXIV) of the four main

divisions of our subject-matter as here grouped. Each vitamin should be studied entirely on its own merits, and without any presuppositions carried over from the study of any other vitamin. The lack of any one vitamin results sooner or later in a nutritional deficiency disease (an *avitaminosis*) which is characteristic for the vitamin in question; and which, if uncomplicated and not allowed to become too severe, can be cured by the giving of the vitamin. In this sense, each vitamin is specific. There is another sense in which the effect of a vitamin is not so specific, though it may still be very important. This is in the further benefit which may be conferred by increased liberality of vitamin value of food above what is needed to protect the body from any symptom of specific deficiency. This added benefit may show itself in what the Journal of the American Medical Association has called "buoyant health as distinguished from merely passable health," and in a better ability to cope with some infections — not always specifically, in the immunological sense, and perhaps not always through the immunological "mechanism" — and yet probably not entirely indiscriminately, nor merely in the vague and obvious sense that a good nutritional condition is better than a poor one. Here again, each vitamin needs to be studied separately upon its own merits.

(5) The four preceding sections indicate a four-fold division of the subject *matter* of our study, in the literal sense of the materials which the food must furnish for the support of the nutritional process. Thus it may be said that any present-day study of nutrition, to be worthy of the name, must stand four-square upon full recognition of energy, protein, mineral elements, and vitamins. Now while each of these is needed as such, and is in that sense independently essential, yet they do not function in isolation; and the interrelationships are so important as to constitute a fifth general aspect of the chemistry of food and nutrition.

Thus, as briefly mentioned above, the food proteins furnish amino acids which serve not only as building materials for the construction of body tissue in the usual sense of the term, but also as precursors from which the body makes the catalysts which expedite the series of reactions involved in bringing the fuel food-stuffs into the service of the energy needs. For instance, glutathione which appears directly to catalyze the oxidation process, thyroxine and adrenine (epinephrine, adrenaline) which function either to expedite the process or to mobilize material for it, insulin which

facilitates some preparatory reaction, and the typical hydrolytic enzymes which bring the organic foodstuffs into forms upon which these other catalysts can act — all these now appear to be, in one way or another, derivatives of amino acids such as result from the digestion of food proteins. Or, again, in hemoglobin the body makes itself a substance which is outstanding both as a protein and as an iron compound; and which functions both in carrying oxygen for the reactions involved in the energy exchange, and in maintaining the acid-base balance. And as a further example of important interrelationships, there are those of the calcium, the phosphorus, and the vitamins concerned in the construction and maintenance of good bones and sound teeth.

These are only a few of the many interrelationships already well-recognized to which more will doubtless be added as knowledge of the nutritional process grows. But with something of the order of thirty-odd chemical entities (elements or compounds as the individual case may be) which the food must supply, the theory of combinations and permutations indicates a calculable number of interrelationships far beyond the possibility of study within a human lifetime. Both for practical reasons and in order to make appreciable progress with theoretical concepts, we need to devote our scientific efforts not equally to all interrelationships or to all conceivable questions as to what happens where in the body, but rather to the problems which we have reason to judge are of greatest significance. And this consideration gives the key to what may be considered a sixth major aspect of our study:

(6) The concept or principle of *natural wholes* as bearing upon problems both of food value and of the nutritive welfare of the entire life-cycle or of a family, community, nation, or race through successive generations. This aspect of our study seeks to give due recognition to the point of view that in the chemistry of nutrition our most significant concern is with the nutritional reactions or responses of the body as a whole. As the very word organism implies, the living body is an organized, coordinated whole, which may behave as something more than a mere summation of its parts. It is such a coordinated whole that is to be nourished. Hence the true significance of a question in the chemistry of nutrition is often best judged by tests in which one observes the nutritional reaction or reactions of the living body as a whole; and such have now become practicable through the standardization,

in recent years, of suitable laboratory animals as instruments and reagents of testing and research in this branch of chemistry.

However interesting and important it may be to follow individual chemical substances into individual bodily organs and to trace complicated interrelations among the details of the intermediary processes in nutrition — and it is thus that much of our knowledge has been, and must be, won — we have recently learned the far-reaching importance of remembering also that in nutrition the most conclusive evidence is that obtained from the experiment or closely-reasoned series of experiments, conducted with all possible exactness and the most careful laboratory control, upon the whole animal, often throughout its entire life-cycle, and sometimes even through successive generations.

Such comprehensive methods of experimental appeal to nature, guided by the principle of the whole, may be regarded as completing a six-fold grouping of the major aspects of the present-day study of the chemistry of food and nutrition. These six aspects may also be regarded as six steps in the progress to our present view; or as six pillar concepts upon and around which the chemistry of nutrition is being built.

### **A Few Definitions and Explanations**

The activities upon which the life of the body depends involve a continuous expenditure of energy and also a never-ceasing exchange of material.

Body substances may be and often are drawn upon to meet temporary deficiencies of nutrient intake; but ultimately the body is dependent upon food for the fuel substances (oxidizable organic nutrients) which supply energy; for the substances which make good the exchange of body material; and (either by supplying the actual substances or their precursors) for all those regulatory factors which keep the reactions running at the right rates, and keep the internal conditions of the body within the zones of delicate adjustment which the life process requires.

Thus the chief functions of food are: (1) to yield energy; (2) to build, or to renew, body tissue; (3) to regulate body processes and internal conditions, so as to maintain a right internal environment for life.

The changes which take place in the foodstuffs, after they have been absorbed from the digestive tract, are included under the

general term *metabolism*. Although the chemical changes and the energy transformations are of course inseparable, it is more or less customary to speak of the metabolism of matter and the metabolism of energy, and to regard the extent of the metabolism of any material substance as measured by the amount of its end products eliminated, and the extent of the energy metabolism as measured by the amount of heat, or of heat and external muscular work, which the body has disbursed.

The chemical factors of an adequate food supply may now be summarized as follows: (1) sufficient of the organic nutrients in digestible forms to yield the needed energy; (2) protein, sufficient in amount and appropriate in kind to yield enough of each of the needed amino acids; (3) adequate amounts and proper proportions of the various mineral elements needed; (4) sufficient of each of the essential vitamins.

There may or may not be, in the case of any given nutrient, a considerable difference between the merely adequate and the optimal intake. This is a question for experimental investigation with reference to each nutrient separately on its own merits. And in such a study the possible influence of variations in the intakes of other nutrients should also be considered.

In attempting to give in the following pages a general view of the chemistry of food and nutrition it has seemed best to discuss first, the chemical nature and nutritive functions of the carbohydrates, fats, and proteins; second, the nutritive requirements of the body in terms first of energy, then of protein; third, the inorganic or mineral elements in food and nutrition; and fourth, the vitamins. In general this permits us to progress from the topics which are more familiar to those which are newer or less well worked out.

It is important to keep clearly in mind the fact that the newer knowledge of nutrition supplements but does not supplant the knowledge which had been gained before vitamins were discovered or the importance of the mineral elements was appreciated.

It is significant that it is Lavoisier, the chemist who first insisted effectively upon making chemical work quantitative and chemistry an exact (as well as a natural) science, who is now considered the father of the science of nutrition. He showed the similarity between the oxidation of organic substance in the body and the burning of a fuel in a flame; and he made many quantitative

experiments upon the rate of oxidation in the body under various conditions. The present-day study of the energy metabolism thus owes its origin to Lavoisier.

Both from the chemical and from the physiological point of view, the term *nutrition* may be used in a very broad sense. Mendel employed the title *Nutrition: The Chemistry of Life*; and, physiologically, the whole state of well-being of the living organism is sometimes spoken of as nutritional.

In the pages which follow we shall be content to deal with nutrition in the somewhat more specific sense of the life processes as chemically connected in known and fairly direct ways with the actual nutrients supplied by the food; but even this will be found, on the one hand, to involve both structural organic and physical chemistry and, on the other hand, to have many far-reaching relationships to the chemical control of the life process and to the resulting health and well-being of the individual and the race.

Present-day science is strongly imbued with the concept that we live in a dynamic world, and that the chief significance of things is to be found not through static descriptions but through the study of their functioning. Our present study will include but little of the descriptive or structural chemistry of the substances found in food; while the chief emphasis will be upon the nutritional functions of these substances. For nutrition is the functional aspect of food chemistry and therefore its dominant aspect from the point of view of present-day science.

### **General Knowledge of Organic Chemistry Presupposed**

In order that the text which follows may be kept within the modest size appropriate to its main purpose, and at the same time maintain a broad viewpoint, the plan throughout is to refrain from repeating here any appreciable amount of the present-day subject-matter of courses and textbooks in general organic chemistry. It is presumed that the student will be prepared to refresh or strengthen his acquaintance with general organic chemistry by turning to its modern textbooks in case of need.

## CHAPTER II

### CARBOHYDRATES

#### Introduction

OF THE constituents of the ordinary mixed food of man the carbohydrates are usually the most abundant and the most economical sources of energy. They are also considered to be the first of the three great groups of organic foodstuffs to be formed by synthesis from simple inorganic substances in plants: "in the long run, all the energy of living matter comes from them." The synthesis of carbohydrates in nature is therefore a logical starting point for the study of the organic foodstuffs.

Green leaves utilize the radiant energy of sunshine to bring about the process of *photosynthesis* in which organic compounds such as sugars and starch are synthesized from carbon dioxide and water. Thus is "bound" or "stored" the energy which later becomes available when any direct or indirect product of photosynthesis is utilized as a fuel foodstuff in the body. Space does not permit a description of the photosynthetic process here; for it is complicated, both as to the chemical substances involved, and in that it appears to be essentially connected with the physiological processes of respiration and metabolism in the plant. The chemistry of plant physiology is very interesting and important, but lies outside the scope of our present study.

Hence we here review the few carbohydrates most important in food and nutrition without attempting the discussion of their formation in nature.

#### Monosaccharides

The monosaccharides are all soluble, crystallizable, diffusible substances, unaffected by digestive enzymes. All of the four with which we are here concerned — glucose, fructose, galactose, and mannose — are utilized for the production of glycogen in the animal body and the maintenance of the normal glucose content

of the blood; but the individual members of this group differ in natural occurrence, and in some details of nutritional relationship.

**Glucose** (*d*-glucose, dextrose, grape sugar, corn sugar, starch sugar, diabetic sugar) is widely distributed in nature, occurring in the blood of all animals in small quantity;\* and more abundantly in fruits and plant juices, where it is usually associated with fructose and sucrose. It is especially abundant in grapes, of which it often constitutes 20 per cent of the total weight or more than half of the solid matter. Sweet corn, onions, and unripe potatoes are among the common vegetables containing considerable amounts of glucose.

Glucose is also obtained from many other carbohydrates by hydrolysis either by acids or by enzymes as in natural digestion and thus becomes the principal form in which the carbohydrate of the food enters into the processes of nutrition. In the healthy body the glucose of the blood is constantly being burned and replaced. Ordinarily any surplus of glucose absorbed from the digestive tract is converted into glycogen which, as described beyond, is readily reconvertible into glucose. Thus, while other carbohydrates occur in food in greater quantity, glucose occupies a very prominent place, partly because it is so widely distributed in both plants and animals, and partly because it is the form in which most of the carbohydrate material of the food comes actually into the service of the body tissues. It is estimated that (under ordinary conditions) over half the energy manifested in the human body is in this sense derived from the oxidation of glucose.

It is not to be inferred from the foregoing statement that the body obtains the energy of the glucose by oxidizing it directly as such. There is abundant evidence of an *intermediary metabolism*; but conflicting views as to its details.

The chemistry of glucose is further reviewed in considering its relations to fructose and galactose in the following paragraphs.

**Fructose** (*d*-fructose, fruit sugar, levulose) occurs with more or less glucose in plant juices, in fruits, and especially in honey, of which it constitutes about one half the solid matter. It results in equal quantity with glucose from the hydrolysis of cane sugar and in smaller proportion from some other less common sugars. Fruc-

\* It is convenient to remember the glucose as constituting about one tenth of one per cent of the blood, but the normal average is slightly less than has been commonly estimated in the past, there being also present small amounts of other substances which react much like glucose to simple analytical testing.



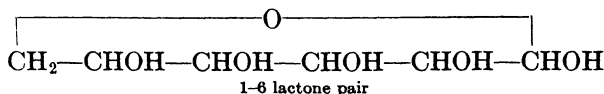
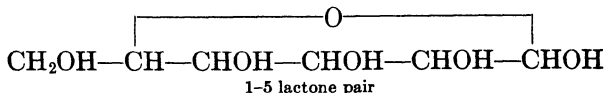
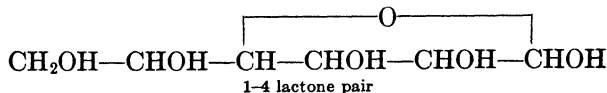
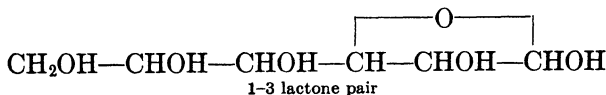
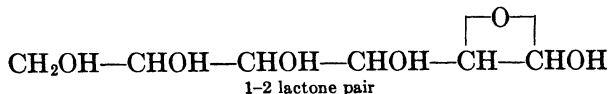
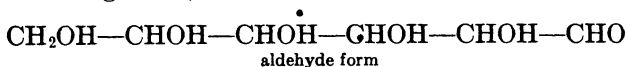
tose serves, like glucose, for the production of glycogen. Glucose and fructose are convertible, either one into the other, under the influence of very dilute alkalies. It is not surprising, therefore, that fructose should be converted in the liver into glycogen, which on hydrolysis yields glucose.

**Glucose and fructose.** A convenient chemical explanation of the mutual interconvertibility of glucose and fructose is that the transformation occurs through the intermediate formation of a di-enol as now commonly shown in textbooks of organic and physiological chemistry.

**Cyclic ("oxygen bridge," lactone, lactal) forms of glucose.** At any given time the glucose present in a solution doubtless exists in *at least* three forms, each of at least one lactone pair in equilibrium with a little of the open chain aldehyde form:

$\alpha$ -glucose (cyclic)  $\rightleftharpoons$  open chain aldehyde  $\rightleftharpoons$   $\beta$ -glucose (cyclic).\*

For the sake of compactness, the possible pairs of lactones (lactals) may be indicated semi-structurally, without stereochemical arrangement, as follows:



\* Cf. Conant's *The Chemistry of Organic Compounds*, 1939 Edition, pp. 307-311.

Of the five structurally possible lactone pairs, two are well established. These are the 1-4 lactone (*furanose*), and the 1-5 lactone (*pyranose*) forms.

Glucose as made in the laboratory, and commercial glucose which is, a product of acid hydrolysis of starch, is chiefly in the form of the 1-5 lactone pair; while glucose in nature presumably is chiefly in the form of the 1-4 lactone pair. Certainly both these forms exist and both the 1-4 and the 1-5 formulas are "right." Probably the above expression of equilibrium stands equally for the *furanose* and *pyranose* forms; and if each is in equilibrium with the aldehyde form they are thus at least indirectly in equilibrium with each other. Hence when we say that the glucose produced *in vitro* is principally the 1-5 form (*pyranose*) and that of nature is principally the 1-4 form (*furanose*), it might be more strictly correct to say that it is chiefly the 1-5 form which enters into or emerges from the familiar *in vitro* reactions, while it is chiefly as the 1-4 form that glucose undergoes its well known nutritional reactions such as the transformation of glucose into galactose radicles in the synthesis of lactose in the mammary gland and the breaking of glucose phosphate into three-carbon intermediates when glucose is metabolized in the tissues generally.

The structural chemistry and interrelationships of the sugars, if not already familiar to the reader, may readily be consulted in textbooks of organic and physiological chemistry.

Bodansky (1938, p. 43) cites Levene as authority for the view that glucose solutions may contain all of the theoretically possible cyclic structures (lactone forms) in equilibrium with each other. The 1-5 form is believed to exist in larger proportion, but under the influence of agents which act specifically upon *any* one form, this form may enter into reaction; and as it reacts and so is removed from the equilibrium the other forms are changed into it. Thus the products may show which form entered predominantly into reaction under the given circumstances, rather than which form was present in largest amount in the original glucose solution. This view should avoid the confusion sometimes caused by the fact that certain reactions *in vitro* indicate chiefly the 1-5, and certain nutritional reactions chiefly the 1-4 form.

**Galactose** results (along with glucose) from the hydrolysis of milk sugar, either by acid or by digestive enzyme, and appears, like glucose and fructose, to promote the formation of glycogen

in the animal body. If glucose and galactose be represented by their (full structural) 1-4-lactone formulas it will be seen that a very direct stereochemical relationship exists between them so that the fact just noted that galactose goes to form the same glycogen in the body as is formed from glucose, and the further fact that galactose is evidently formed from glucose in the mammary gland, both become readily intelligible. For the difference between glucose and galactose as thus represented lies only in the spatial relation of the oxygen bridge to the rest of the molecule, and this is evidently changed in the mammary gland with resulting transformation of glucose into galactose which then combines with some of the remaining glucose to form lactose. The transformation probably occurs through the enzymic action of something in the gland cell which first forms a combination with glucose and then splits off galactose, the stereoisomeric shift of the oxygen bridge occurring in the course of the formation and subsequent cleavage of the complex intermediate compound which the sugar radicle forms with the enzyme molecule — this latter being itself doubtless a very complex and optically active substance.

Polymeric anhydrides of galactose, known as galactans, occur quite widely distributed in plants; and galactosides, which are compounds containing galactose in chemical combination with radicles of other than carbohydrate nature, are constituents of the brain and nerve tissues. Recently, galactose has been recognized as a minor constituent of several proteins.

**Mannose** also, although not prominent in the carbohydrate food-stuffs, has recently been reported as occurring in several proteins.

### Disaccharides

The three disaccharides here considered are *dihexoses* or *hexobioses* of the formula  $C_{12}H_{22}O_{11}$ , and are crystallizable and readily diffusible. Sucrose crystallizes anhydrous; maltose and lactose each with one molecule of water, which can be removed by drying at temperatures of 100° and 130° respectively. They are soluble in water; less soluble in alcohol. Lactose is much less soluble than sucrose and maltose. These disaccharides are important constituents of food and are changed to monosaccharides during the process of digestion.

**Sucrose** (saccharose, cane sugar) is widely distributed in the vegetable kingdom, being found in considerable quantity, gen-

erally mixed with glucose and fructose, in the fruits and juices of many plants. The commercial sources of sucrose are the sugar beet, the sugar and sorghum canes, the sugar palm, and the sugar maple; but many of the common fruits and vegetables contain notable amounts. For example, sucrose is said to constitute at least half the solid matter of pineapples and of some roots such as carrots.

The leading source of sucrose is the sugar cane, the second is the sugar beet, and all others are of only local or insignificant commercial importance compared with these. Needless to say, there is a large literature on sugar production, sugar refining, and the sugar trade. Besides the special books and journals devoted to sugar, several of the handbooks on industrial chemistry have sections on the sugar industry, and a chapter on the subject is included in the writer's *Food Products*.

The per capita consumption of sugar per year in the United States has increased from 68 pounds in 1898-1902 to 74 in 1903-07, to 79 in 1908-12, to 83 in 1913-17, to 94 in 1918-22, to 109 in 1923-27. Thus we came to take about one seventh of our total calories in the form of this food, which correspondingly displaces one seventh of the protein, the mineral elements, and the vitamin values, which we would otherwise get in eating to meet our energy requirement. Is it a matter of indifference to have our intakes of the other factors of our nutrition thus reduced? Or would our best health interests require a food supply richer in some at least of the mineral elements and vitamin values?

Would we be better off if a part of the producers' land and labor, and of the consumers' money, now devoted to sugar, were devoted instead to increasing the production and consumption of foods which together with their calories furnish us also other nutrients needed to balance our dietary? Facts bearing upon such questions will be developed in many of the chapters which follow.

On hydrolysis, a molecule of sucrose yields one molecule each of glucose and fructose. These sugars all rotate the plane of polarized light: sucrose and glucose to the right (+), and fructose to the left (-). The terms *dextrose* and *levulose*, synonyms for glucose and fructose respectively, arose from this behavior of the sugars in rotating the plane of polarized light to the right and left. Since at ordinary temperatures the fructose rotates more strongly to the left than the glucose does to the right, the result of the

hydrolysis of sucrose is to change the sign of rotation from  $+$  to  $-$ . For this reason the hydrolysis of cane sugar is often called *inversion*, and the resulting mixture of equal parts of glucose and fructose is known as *invert sugar*.

Sucrose is very easily hydrolyzed either by acid or by the *sucrase* (*invertase* or "inverting" enzyme) of yeast or of intestinal juice. So far as known, neither the saliva nor the gastric juice contains any enzyme capable of hydrolyzing cane sugar, and any hydrolysis of sucrose which takes place in the stomach is believed to be due to the presence of hydrochloric acid or to the regurgitation of intestinal juices into the stomach, which may occur more commonly than is generally realized. In the intestine, sucrose is split by the sucrase of the intestinal juice, and the resulting glucose and fructose are absorbed into the portal blood.

**Lactose** (milk sugar) occurs in the milk of all mammals, constituting usually from 6 to 7 per cent of the fresh secretion in human milk and 4.5 to 5 per cent in the milk of cows and goats. At the time of parturition or if the milk is not withdrawn from the udder, some lactose may occur in the urine. If in such a case the mammary glands are removed, the percentage of glucose in the blood increases, and glucose (but no lactose) may appear in the urine. These observations (due chiefly to experiments made by Moore and Parker at the suggestion of Schaefer and of Lusk) indicate that lactose is formed in the mammary gland and probably from the glucose brought by the blood. The chemistry of the process has been discussed briefly in the section on galactose above. For fuller discussion and further experimental study see Watkins (1928) and Winter (1931).\*

Lactose is less sweet and much less soluble than sucrose, dissolving only to the extent of about 1 part in 6 parts of water. When hydrolyzed either by heating with acid or by an enzyme, such as the *lactase* of the intestinal juice, each molecule of lactose yields one molecule of glucose and one of galactose. Hence the lactose eaten is absorbed not as such, but as a mixture of equal parts glucose and galactose. Herter found lactose to be less subject to fermentation in the stomach than is sucrose. Also, because of the much lower solubility, there is less danger of direct irritation of the stomach membrane by lactose than by sucrose. Mathews

\* References for suggested reading, whether specifically cited in the text or not, are given at the ends of the respective chapters.

has suggested that the occurrence in milk of lactose, a sugar having the galactose radicle, may be of special significance as a source of material for the synthesis of the galactosides of the brain and nerve tissues of the rapidly growing young mammal. Furthermore, much recent research, summarized and reaffirmed by Associates of Rogers (1935), has shown that the taking of liberal quantities of lactose in the food is for many people a very important aid to the maintenance of good intestinal conditions, because the lactose is especially favorable to the development of desirable types of intestinal bacteria, notably the *Bâcillus* (*Lactobacillus*) *acidophilus*.

In order that this latter advantage may be had along with greater solubility and apparent sweetness for those who desire it, *beta-lactose* \* has been studied scientifically and made commercially available. As yet the consumption of lactose of both forms in this country is far less than the amount which its dairy industry could very readily supply.

**Maltose** (malt sugar) is formed from starch by the action of diastatic enzymes (amylases) and is therefore an important constituent of germinating cereals, malt, and malt products. It is also formed as an intermediate product when starch is digested in the human or other animal body, or hydrolyzed by boiling with dilute mineral acid as in the manufacture of commercial glucose.

In animal digestion, maltose is formed by the action of the ptyalin of the saliva (salivary amylase) or of the amylopsin of the pancreatic juice (pancreatic amylase) upon starch or dextrin. The maltose-splitting enzyme of the intestinal juice readily hydrolyzes maltose to glucose. Maltose is also readily and completely hydrolyzed by boiling with dilute mineral acids. In either case each molecule of maltose yields two molecules of glucose.

While it is probable that little if any maltose is absorbed as such from the digestive tract under ordinary conditions, it is possible that such absorption may occur and that maltose as such may play a part in the normal carbohydrate metabolism; for when injected into the blood it appears to be utilized to better advantage than is either sucrose or lactose when thus injected, and it may be obtained from glycogen by the action of diastatic enzymes in much the same way as from starch and dextrin.

\* Lactose, glucose- $\beta$ -galactoside, exists in  $\alpha$ - and  $\beta$ -forms corresponding to  $\alpha$ - and  $\beta$ -glucose. The ordinary "milk sugar" of commerce is  $\alpha$ -lactose. However by allowing solutions of lactose to crystallize above 90°, the  $\beta$ -isomer is obtained.

### Polysaccharides

The polysaccharides are all insoluble in alcohol. Some dissolve in water in the sense that they form colloidal dispersions which will pass through filter paper; some swell and become gelatinous; some are apparently unchanged. The members of greatest importance in nutrition are starch and glycogen, the typical reserve carbohydrates of plants and animals respectively, and the dextrans which are intermediate products in the hydrolysis of starch.

**Starch**, approximately  $(C_6H_{10}O_5)_x$ , is the form in which most plants store the largest part of their carbohydrate material, and is of great importance as a constituent of many natural foods and as the source of dextrans, maltose, glucose, and many fermentation products. Starch is found stored in the seeds, roots, tubers, bulbs, and to some extent in the stems and leaves of plants. It constitutes one half to three fourths of the solid matter of the ordinary cereal grains and at least three fourths of the solids of mature potatoes.

Unripe apples and bananas contain much starch which is to a large extent changed into sugars as these fruits ripen; while, on the other hand, young tender corn (maize) kernels and peas contain sugar which is transformed into starch as these seeds mature.

Starch granules are scarcely affected by cold water; on warming they absorb water and swell. Finally the starch passes into a condition of colloidal dispersion or semi-solution, "starch paste." Starch which has been heated in water (either admixed or naturally present with the starch as in a potato) until the granules are ruptured and the material more or less dispersed is very much more rapidly hydrolyzed by digestive enzymes than is raw starch. The starch-digesting enzymes (*amylases*) of the saliva and pancreatic juice change starch through dextrin to maltose; and (as noted above) a digestive ferment of the intestinal juice changes the maltose to glucose. Acid hydrolysis also changes starch through dextrin and maltose to glucose.

Thus far we have followed the usual custom of speaking of starch as if it were a single substance of purely carbohydrate nature. Actually, however, the strictly carbohydrate material of the starch is combined with a very minute proportion of one or more acid radicles, sometimes of fatty acid and sometimes

containing phosphorus. Also starch yields, even under such treatment as would not be expected to disrupt a chemical compound, two substances:  $\alpha$ -amylose (also called amylopectin) which contains the small amount of non-carbohydrate material, and which forms on heating in water a viscous opalescent paste; and  $\beta$ -amylose (also called amylose) which forms when heated in water a clear limpid solution which gives a pure blue color with iodine. The starch-digesting enzymes hydrolyze both  $\alpha$ -amylose and  $\beta$ -amylose, but not necessarily at the same rate.

"Soluble starch," largely used for laboratory experiments, is commonly prepared by soaking raw potato starch in cold hydrochloric acid (about 7 per cent HCl) for several days, and then washing with cold water, or by treating starch with alkali under very carefully regulated conditions.

**Dextrins**, approximately  $(C_6H_{10}O_5)_x$ , or  $(C_6H_{10}O_5)_x \cdot H_2O$ , are formed from starch by the action of enzymes, acids, or heat. The term *dextrin*, even if used in the singular, must be understood as designating a group rather than an individual substance. Small amounts of dextrin are found in normal, and larger amounts in germinating, cereals. Malt diastase, acting for some time upon starch in fairly concentrated solution, yields usually about one part of dextrin to four of maltose. Dextrins thus formed by hydrolysis are sometimes called *hydrolytic dextrins*. Commercial dextrin, the principal constituent of "British gum," is obtained by heating starch, either alone or with a small amount of a dilute, or of a weak, acid. The dextrins formed essentially by dry heating are sometimes called *torrefaction dextrins*.

The dextrins are much more soluble than the starches; and dextrin molecules, while doubtless very large and complex, are probably much smaller in size than the starch molecules from which they are derived.

The digestion of dextrin has already been mentioned in connection with that of starch, both saliva and pancreatic juice forming dextrin during the digestion of starch and acting upon it with the production of maltose. Complete hydrolysis of dextrin, as by boiling with acid, yields glucose as the final product.

The work of Rettger indicates that dextrin is, like lactose, a favorable medium for the nourishment of the *Lactobacillus acidophilus* in the digestive tract, so that liberal proportions of dextrin and lactose in the food are conducive to a good intestinal hygiene.



**Glycogen**,  $(C_6H_{10}O_5)_x$ , plays much the same rôle in animals which starch plays in plants, and is sometimes called *animal starch*. It is a white, amorphous powder, odorless and tasteless, which swells up and apparently dissolves in cold water to an opalescent colloidal dispersion which is not cleared by repeated filtration, but loses its opalescence on addition of a very small amount of potassium hydroxide or acetic acid. Water solutions (dispersions) of glycogen are readily precipitated by alcohol. When treated with iodine they react yellow-brown, red-brown, or deep red. Complete hydrolysis of glycogen yields glucose only, as an end-product.

Glycogen occurs in the lower as well as the higher animals, and in nearly all parts of the body, but reaches its highest concentration in the liver. The amount of glycogen in the liver depends to a great extent upon the condition of nutrition of the animal. In the average of seven experiments by Schöndorff in which dogs were fed for the production of as much glycogen as possible, 38 per cent of that found was in the liver, 44 per cent in the muscles, 9 per cent in the bones, and the remaining 9 per cent in the other tissues of the body. But the distribution of glycogen in the body as shown by these experiments was quite variable, even among animals of the same species which had been fed in the same way. It is well known, too, that some species store glycogen in their muscles to a greater extent than others. The storage of glycogen in the body is promoted by rest as well as by liberal feeding, and stored glycogen is used up rapidly during active muscular work.

**Cellulose and hemicelluloses** are polysaccharides still less soluble or less readily hydrolyzed than those above described. They contribute little if anything to the strictly nutritive values of foods, but their presence in proper proportion in the diet as a whole is favorable to the mechanics of digestion and the hygiene of the digestive tract. All such material not dissolved by the digestive enzymes is sometimes called "roughage"; but more accurately speaking it is only some forms of fiber which are rough, while other forms of cellulose and nearly all forms of hemicellulose are relatively soft and smooth, yet contribute a desirable *bulk* to the food mass and its undigested residue. Every normal dietary should contain enough indigestible fiber (chiefly celluloses and hemicelluloses) to give the digestive canal "its daily scrubbing";

but to what extent this should be real roughage, and to what extent the soft smooth forms of cellulose and hemicellulose should be preferred, is a matter which may differ largely with individuals. An amount of real roughage (or a degree of real roughness) which is advantageous to one person may be excessive for another. In a population of many millions there very likely may be a considerable number of people who have treated themselves to too much roughage; and there may at the same time be an equal or larger number of people whose ordinary food habits give them less bulk of undigested residue than is best. In cases of doubt, one may carefully experiment (1) with increased intakes of soft cellulose and hemicelluloses as in ordinary fruits and vegetables, and (2) with a rougher form of fiber as in bran. The effects of different chemical components of vegetable fiber are being studied. There is also growing recognition of the value of liberal amounts of fruit juices in promoting intestinal regularity.

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## CHAPTER III

### FATS AND LIPOIDS: LIPIDS (LIPINS)

BLOOR (1925) defines lipids as: "Substances having the following characteristics: (a) Insolubility in water and solubility in the fat solvents, such as ether, chloroform, benzene. (b) Relationship to the fatty acids as esters, either actual or potential. (c) Utilization by living organisms." He classifies them as follows:

#### Bloor's Classification of Lipids

**Simple lipids.** Esters of the fatty acids with various alcohols.

Fats — esters of the fatty acids with glycerol.

Waxes — esters of the fatty acids with alcohols \* other than glycerol.

**Compound lipids.** Esters of the fatty acids containing groups in addition to an alcohol and fatty acid.

Phospholipids — substituted fats containing phosphoric acid and nitrogen — lecithin, cephalin, sphingomyelin.

Glycolipids — compounds of the fatty acids with a carbohydrate and containing nitrogen but no phosphoric acid — cerebro-sides.

Aminolipids, sulfolipids, etc. — groups which are at present not sufficiently well characterized for classification.

**Derived lipids.** Substances derived from the above groups by hydrolysis.

Fatty acids of various series.

Sterols — mostly large molecular alcohols, found in nature combined with the fatty acids and which are soluble in the fat solvents — cholesterol ( $C_{27}H_{45}OH$ ), myricil alcohol ( $C_{30}H_{61}OH$ ), cetyl alcohol ( $C_{16}H_{33}OH$ ), etc. [More recently the term sterols has generally been restricted to complex hydroaromatic secondary alcohols such as cholesterol and ergosterol, cf. Bills, 1935.]

\* In the familiar waxes, these are alcohols of high molecular weight; and in most cases are "monatomic," i.e., with only one hydroxyl.

Bloor comments on the above definitions as follows:

"Almost all the known lipids are found in living organisms, so that the general characteristics of the group are in the main those of naturally occurring substances. But such substances as have been produced synthetically behave, as far as is known, like the natural ones, and there is no reason to believe that the characteristics of the group will have to be altered to suit synthetic members. . . .

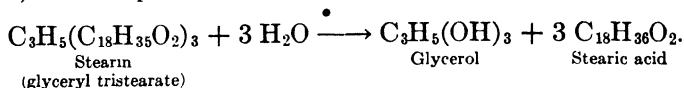
"The most general characteristic of the group is the solubility in fat solvents such as ether, chloroform, benzene, as contrasted with the insolubility in water. This of itself is sufficient to set it off from the other great groups of biological substances — the carbohydrates, proteins, and mineral salts. The property is not absolute, since certain members of the group such as the lecithins form dispersions on mixing with water, which are at least colloidal and may approach true solubility. On the other hand, many members of the group are not soluble in all fat solvents. For example, most of the lecithins are insoluble in acetone, the cephalins are mainly insoluble in alcohol, while sphingomyelin and the cerebrosides are difficultly soluble in ether.

"In order to exclude organic compounds which have no biochemical relationship to the fats or fatty acids, but which from their solubilities alone would be included in the group, the limitations in (b) and (c) have been applied. The substances included in the group must be either ester-like combinations of the fatty acids or capable of forming such combinations, and they must be capable of performing some useful functions in living organisms."

*Many hydrocarbons* are also now known to be of significance to life processes and may, therefore, be added to the foregoing classification. As a few examples illustrating a wide range of biological significance, the carotenes, the terpenes, and the carcinogenic hydrocarbons may be mentioned. The carotenes (and cryptoxanthin) as precursors of vitamin A are considered in Chapter XXII. The other biologically significant hydrocarbons do not seem to fall within the scope of this book but may be readily studied, if desired, in present-day textbooks of physiological or biological chemistry, or such textbooks of organic chemistry as give attention to all classes of naturally occurring organic compounds.

### Fats

The fats are almost as widely distributed in nature as the carbohydrates, and constitute a much more concentrated form of fuel to supply energy in nutrition. Fats are glyceryl esters of fatty acids, and since glycerol is a triatomic alcohol and the fatty acids are monobasic, a normal glyceride is a triglyceride and on hydrolysis yields three molecules of fatty acid and one molecule of glycerol. Thus, for example:



When, as is usual, the splitting of the fat is brought about by means of an alkali, the corresponding products are glycerol and three molecules of the alkali salt of the fatty acid; and since alkali salts of the fatty acids are commonly known as soaps, this reaction is usually called the *saponification* of the fat.

The fats are, as implied in the above definition, a structurally distinct group of chemical compounds, and the term applies equally to the solid and the liquid members of this group. As a matter of convenience, however, the liquid fats are often called *fatty oils*. The fatty oils are also sometimes called *fixed oils*, since a spot made by dropping a fatty oil on paper cannot be removed by drying (as can a volatile oil), nor by washing with water (as can glycerol).

The fat of a food (with more or less of the phosphatides and sterols) may be separated from the other chief components by drying the food and extracting the dry material with pure ether. After the fat has been completely dissolved away from the other constituents of the food, it can be recovered from the solvent by evaporating the latter at a relatively low temperature. This is the method commonly used to estimate the percentages of fat in foods and to obtain small portions of fat for examination. It must be noted, however, that the fat thus obtained is not always pure in the sense of consisting entirely of substances meeting the definition of fat as given above; but will contain, along with the fat, any other ether-soluble substances which were present in the food, and may contain substances which, while not appreciably soluble in ether alone, are dissolved by the mixture of ether and fat. It is, therefore, somewhat more accurate to speak of the ma-

terial extracted by ether as *ether extract* rather than as *fat*, and it will be found so designated in some statements of analytical results.

The food fats of commerce have been separated from the materials in which they naturally occurred, not usually by solvents as above described, but by mechanical means such as churning (butter) or pressing (olive or cottonseed oil); but even in this case the naturally occurring fat-soluble substances will still remain dissolved in the separated fat. Some of these fat-like and fat-soluble substances, although occurring only in small quantities, have very important functions in nutrition. We shall have occasion to study them in that connection later.

Shortages of food fats during the World War of 1914-18 brought about an increased appreciation of the food values of certain fats which previously had been applied chiefly to industrial uses.

The glycerides of any common natural fat, with the probable exception of butter, would if obtained absolutely pure be colorless, tasteless, and odorless. The colors, tastes, and odors of natural fats are, therefore, ordinarily due to substances present in small amount which might be removed by refining processes; while the permanent, quantitative differences among the fats are to be accounted for by the kinds and the amounts of the fatty acids which enter into the composition of the glycerides.

Some of the fatty acids have been (and so their glycerides could be) produced artificially, starting with petroleum hydrocarbons. All such cases of laboratory syntheses of natural substances are scientifically significant; but their economic significance is often much less than is expected. In the long view it seems entirely probable, as Hilditch has explained, that the production of fats as crops, the amounts of which can be increased both by intensive development and by extension of area, and which as crops are recurrently produced anew, will outrank in permanent importance any industrial synthesis of fats which can now be foreseen: partly because the starting point of the synthetic process is something which, even if now abundant, is limited in quantity and not promptly reproducible like a crop; and also because the particular fatty acids and combinations thereof demanded for food purposes will probably continue to be more expensive to produce synthetically than by natural and cultivated growth of plants and animals, especially as science is learning to improve



plant and animal production both qualitatively and quantitatively through the combined developments of genetics and of plant and animal nutrition.

### Fatty Acids

The greater number of the naturally occurring fatty acids belong to a few homologous series. The series to which stearic acid belongs may be represented by the general formula,  $C_nH_{2n}O_2$ . The members of greatest importance are as follows:

#### ACIDS OF THE SERIES $C_nH_{2n}O_2$

*Butyric acid*,  $CH_3(CH_2)_2COOH$ , occurs as glyceride to the extent of about 5 to 6 per cent in butter and in very small quantities in a few other fats.

*Caproic acid*,  $CH_3(CH_2)_4COOH$ , is obtained from goat and cow butter and coconut fat.

*Caprylic acid*,  $CH_3(CH_2)_6COOH$ , is obtained from coconut oil, goat and cow butter, and human fat.

*Capric acid*,  $CH_3(CH_2)_8COOH$ , is obtained from coconut oil, goat and cow butter, and the fat of the spice bush.

*Lauric acid*,  $CH_3(CH_2)_{10}COOH$ , occurs abundantly as glyceride in the fat of the seeds of the spice bush, and in smaller proportions in butter, coconut fat, palm oil, and some other vegetable oils.

*Myristic acid*,  $CH_3(CH_2)_{12}COOH$ , is obtained from nutmeg butter, coconut oil, butter, lard, and many other fats, as well as from spermaceti and wool wax.

*Palmitic acid*,  $CH_3(CH_2)_{14}COOH$ , occurs abundantly in a great variety of fats, both animal and vegetable, including many fatty oils, and also in several waxes, including spermaceti and beeswax.

*Stearic acid*,  $CH_3(CH_2)_{16}COOH$ , is found in most fats, occurring more abundantly in the solid fats and especially in those having high melting points.

Butyric acid is a mobile liquid, mixing in all proportions with water, alcohol, and ether, boiling without decomposition, and readily volatile with steam. With increasing molecular weight the acids of this series regularly show increasing boiling or melting points, decreasing solubility, and loss of volatility with steam. For ordinary temperatures the dividing line between liquids and solids falls at about caproic acid. Stearic acid is a crystalline solid, insoluble in water, and only moderately soluble in alcohol and ether.

## UNSATURATED FATTY ACIDS

These are unsaturated compounds in that each molecule contains one or more ethylene linkage or "double bond," and can take up halogen by addition to form a saturated compound; or, with a suitable catalyst, hydrogen may be added (hydrogenation). The relative number of double bonds is measured analytically by determining the percentage of iodine which the fat or fatty acid will absorb. This is called the *iodine number*. Thus pure oleic acid (mol. wt. 282) absorbs 2 atoms of iodine, giving an iodine number of 90; pure linoleic acid (the corresponding acid of the  $C_nH_{2n-4}O_2$  series) would absorb 4 atoms of iodine to the molecule, giving an iodine number about twice as great; and so on. These unsaturated acids have, as a rule, much lower melting points than the saturated acids containing the same number of carbon atoms. The glycerides show correspondingly lower melting points than those of the saturated fatty acids and are therefore found more largely in the soft fats and the fatty oils. Such soft fats or fatty oils can be hardened to any desired consistency (up to that of stearin) by hydrogenation, which changes the unsaturated fatty acid radicles into the corresponding members of the more saturated series. This process has been enthusiastically developed commercially and large quantities of oil are now hydrogenated to the consistency of lard substitutes. It remains to be determined how far this is at the expense of the special nutritional value which food fats owe to the presence of some of the more highly unsaturated fatty acid radicles.

*Oleic acid*,  $C_{18}H_{34}O_2$ , occurs as glyceride in nearly all fats and fatty oils and is by far the most important member of the series. Many of the typical oils of both animal and vegetable origin, such as lard oil and olive oil, consist largely of glycerides containing oleic acid radicles.

*Erucic acid*,  $C_{22}H_{42}O_2$ , has long been known as obtainable from the seed oils of cruciferous plants, such as the commercial fatty oils of rapeseed and of mustardseed. More recently it has been found also in marine animal oils (Hilditch).

Oleic and erucic acids are the best-known members of the series  $C_nH_{2n-2}O_2$ .

Acids of the series  $C_nH_{2n-4}O_2$ ,  $C_nH_{2n-6}O_2$ , and  $C_nH_{2n-8}O_2$  have now been found to occur as glycerides in many fats. *Linoleic acid*,

$C_{18}H_{32}O_2$ , and *linolenic acid*,  $C_{18}H_{30}O_2$ , take their names from having been first found in linseed oil; *arachidonic acid*,  $C_{20}H_{32}O_2$ , occurs in the lipids of the brain, liver, and other organs, and of egg yolks. These are but examples; doubtless many other fatty acids of these series occur in natural fats.

It is now generally regarded as established that some unsaturated fatty acid or acids is or are *nutritionally essential* in the sense in which this term has long been applied to some of the amino acids in connection with protein metabolism, *i.e.*, these acids either are not synthesized in the body or not rapidly enough to meet its needs, so that they must be furnished in some form in the nutriment. Of course these nutritionally essential substances need not exist free in the food: the nutritionally essential amino acids exist in the food chiefly as constituents of food proteins; and the nutritionally essential fatty acid or acids, chiefly as constituents of some of the food fats.

Recognition of the special nutritional importance of some of the more highly unsaturated fatty acids has been followed by reinvestigation of several food fats as to the presence of such acids, with the result that they are now being reported where previously they had escaped attention. Butter fat has been found by Hilditch and Sleightholme (1930) to contain linoleic acid, while Bosworth and Brown (1933) add decenoic ( $C_{10}H_{18}O_2$ ) and tetradeceanoic ( $C_{14}H_{26}O_2$ ), Eckstein (1933) adds linolenic, and Bosworth and Sisson (1934) find that arachidonic acid is also present. See also Green and Hilditch (1935).

### Simple and Mixed Triglycerides

Triglycerides in which the three fatty acid radicles are of the same kind are known as *simple triglycerides*. Tristearin, triolein, tripalmitin, are examples of simple triglycerides. A *mixed triglyceride* is one in which the three fatty acid radicles are not all of the same kind. For example, distearo-olein (having two radicles of stearic and one of oleic acid), dioleo-palmitin (having two of oleic and one of palmitic), and stearo-oleo-palmitin (having one radicle each of stearic, oleic, and palmitic acids), are mixed triglycerides.

Taking account of the structurally possible isomers, it can be calculated, according to the principle of combinations and permutations, that the number of mixed triglycerides may increase rather

rapidly with the number of kinds of fatty acid concerned; *e.g.*, with ten kinds of fatty acid, ten simple triglycerides and 540 mixed triglycerides are possible.

The simple triglycerides corresponding to the common fatty acids are all known; but naturally not all of the many possible mixed triglycerides have been prepared. There is, however, no reason to doubt that the mixed triglycerides compose a large part of most natural fats.

When we consider the important extent to which lipids enter into the composition of body tissues, fluids, and membranes, and modify their properties, we see that the probable existence of large numbers of triglycerides may be of very great significance in connection with the differences in physico-chemical structure and properties which are so influential in determining the transport and utilization of nutritive material in and through the fluids and membranes of the body.

### Formation and Composition of Natural Fats

Fats are formed both in plants and in animals. The conditions which determine fat formation, and the character of the fat formed in different species and under different conditions, are better known than the chemical steps involved in the process. It is hardly necessary to mention the fact that the true fats are composed of the same three chemical elements of which the carbohydrates are composed (carbon, hydrogen, and oxygen) and that since the fats contain less oxygen and more carbon and hydrogen than the carbohydrates, they constitute a more concentrated form of fuel or a more compact and lighter medium for the storage of energy for future use. Also, in the animal body, stored carbohydrate is accompanied by much more water than is stored fat.

Hilditch and others have emphasized the general trend toward higher proportions of unsaturated and then of more highly unsaturated fatty acids in the fats formed in cooler regions, by plants as well as by animals.

#### *Formation of Fat from Carbohydrate*

*In plants* there are many indications of the formation of fat from carbohydrate, as when decrease of starch and increase of fat go on simultaneously in a ripening seed, or when sugars are

found to be constantly brought to a tissue in which fat is forming, and to disappear there as the formation of fat progresses.

*In animals* it is certain that fat may be formed from carbohydrate. From the standpoint of our present knowledge it would seem that the readiness with which farm animals are fattened on essentially carbohydrate food should have been sufficient to convince early observers; but this evidence was at one time overlooked because of the idea, then prevalent, that simpler substances are built up into more complex compounds only in the plant, and not in the animal organism. Later, chemists learned that animals as well as plants may show great synthetic activity and there is now abundant evidence that the animal body synthesizes fat from carbohydrate.

The most obvious method of demonstrating the conversion of carbohydrate into fat is that followed by Lawes and Gilbert. Several pigs of the same litter and of similar size were selected; some were killed and analyzed as controls, while the others were fed on known rations and later weighed, killed, and analyzed to determine the kinds and amounts of material stored in the body. In several cases the amounts of fat stored during such feeding trials were found to have been much larger than could be accounted for by all of the fat and protein fed, so that at least a part, and in some cases the greater part, of the body fat must have been formed from the carbohydrate of the food. Many similar experiments have been made, and the transformation of carbohydrate into fat has been demonstrated by this method in carnivorous as well as herbivorous animals.

It has also been shown that carbohydrates contribute to the production of milk fat. Jordan and Jenter kept a milch cow for fifty-nine days upon food from which nearly all of the fat had been extracted. During this period about twice as much milk fat was produced as could be accounted for by the total fat and protein of the food, and in addition the cow gained in weight and her appearance showed that she had more body fat at the end than at the beginning of the experiment.

Instead of determining directly the fat formed in the animal fed on carbohydrate, the production of fat from carbohydrate may be demonstrated by keeping the animal experimented upon in a respiration chamber so arranged that the total carbon given off from the body may be determined and compared with the

total carbon of the food. If in such a case the body is found (by the methods explained in later chapters) to store more carbon than it could store as carbohydrate or protein, it is safe to infer that at least the excess of stored carbon is held in the form of fat. Many such experiments upon dogs, geese, and swine have shown storage of carbon very much greater than could be accounted for on any other assumption than that a part of the carbon of the carbohydrates eaten remained in the body in the form of fat.

Further evidence of the transformation of carbohydrate into fat in the animal body is obtained from the respiratory quotient, as explained in Chapter VII.

### Composition and Properties of Animal Fat

Usually the nature of the fat found in the body is more or less characteristic of each species or group of closely related species. Herbivora contain as a rule harder fats than carnivora, land animals have harder fats than marine animals, and all warm-blooded animals have fats of higher melting points than those found in fishes. The fats of the adipose tissues of different mammals show little variation from an average of: carbon, 76.5 per cent; hydrogen, 12 per cent; oxygen, 11.5 per cent.

Butter fat differs from body fat in containing fatty acids of lower molecular weight (particularly butyric acid, which is fairly characteristic of butter), and so shows a higher percentage of oxygen and lower percentages of carbon and hydrogen. The most abundant acids of butter fat are, however, palmitic, oleic, and myristic, and the ultimate composition is not very greatly different from that of body fats. A sample of milk fat analyzed by Browne showed 75.17 per cent carbon, 11.72 per cent hydrogen, and 13.11 per cent oxygen. For further studies of milk fat and the influence of food upon it see the work of Hilditch and Sleight-holme (1931), of Eckstein (1933), of Bosworth and Brown (1933), of Bosworth (1934), and of Riemenschneider and Ellis (1936).

### *Food Fat and Body Fat*

In discussing the formation of body fat from carbohydrate it was shown that often the greater part of the fat stored is manufactured in the body from carbohydrate. So striking were the results of some of the experiments demonstrating the synthesis of fat from carbohydrate, that it was even questioned for a time

whether any of the fat deposited in the tissues comes from the fat in the food.

Abundant evidence that food fats may contribute to the store of fat in the body has been obtained by feeding characteristic fats or "tagged" or "tracer" fatty acids and showing that these fats can afterwards be recognized in the tissues of the experimental animals. Experiments of this kind were early made with dogs, using linseed oil, rapeseed oil, or mutton tallow, any of which is distinguishable by the chemical and physical properties of its fatty acids from the fat normally found in the body of the dog. More recently it has become possible to use fatty acids containing deuterium (heavy hydrogen) in experiments of this kind. Schoenheimer and Rittenberg (1935, 1936), starting with linoleic acid, introduced four atoms of deuterium to form stearic acid 6-7-9-10  $d_4$ , then fed this and later determined deuterium in the fats of different parts of the body. Their experiments impressed them with the extent to which the fat fed is carried to the body's fat depots, even when there is no apparent surplus of fat.

The occurrence in the body fat of properties usually characteristic of some particular fat which has been fed has long been known and recognized in establishing standards of purity for fats of animal origin. Thus, the lard obtained from swine which have been fed cottonseed meal shows the characteristic color reactions of cottonseed oil, and more elaborate tests must be made in order to determine whether cottonseed fat has actually been mixed with the lard.

Evidence of the formation of body fat from food fat has been obtained also by experiments upon the total amount of fat formed in the body when the amount and composition of the food eaten was accurately known.

Thus there is abundant experimental evidence that both the carbohydrate and the fat of the food may serve as sources of body fat; and it is known that protein also may contribute to the production of fat in the body, though this may possibly be through the intermediate formation of carbohydrate.

A question naturally arises as to how, if proteins, fats, and carbohydrates of food may all contribute to the production of body fat, the nature of the fat can still be to any significant degree characteristic of the species in which it is found. A partial explanation appears to be furnished by the work of Bloor, who finds that, when the fat of the food has been split to glycerol and fatty acids in the course of digestion and these digestion products are taken up and re-synthesized to fat in the intestinal wall, there

may go into the re-synthesized fat not only the fatty acid radicles of the food fat but also fatty acid radicles formed in the body. These latter, entering into the constitution of the re-formed fat, tend to give it some of the properties characteristic of the species while at the same time some of the characteristics of the food fat may be retained. Much study has been given to the effect of food upon body fat. References to many of the original papers will be found at the end of the chapter.

Limitation of space makes it necessary to confine ourselves here to the one phase of this work which is uppermost in the present discussion. Ellis and Isbell demonstrated anew the direct carrying over of some fatty acid radicles from food to body fat by the finding of linolenic acid in the lard of animals fed soybeans, and of arachidic acid in that of those fed peanuts. Anderson and Mendel developed a method of studying the influence of food upon the quality of the total body fat. Rats were fed a food-mixture in which 40 per cent of the calories were in the form of skimmed-milk powder and 60 per cent in the form of the food fat to be tested. The results as indicated by the iodine numbers of the fats are summarized in Table 1. Here the influence of the food fat upon

TABLE 1. — INFLUENCE OF FOOD FAT UPON BODY FAT  
(Anderson and Mendel)

| FOOD FAT<br>(60% of Total Calories Fed) | IODINE NUMBER<br>OF FOOD FAT | IODINE NUMBER<br>OF BODY FAT |
|---|------------------------------|------------------------------|
| Soybean oil . . . . .                   | 132.3                        | 122.5                        |
| Corn (maize) oil . . . . .              | 124.3                        | 114.2                        |
| Cottonseed oil . . . . .                | 108.1                        | 107.4                        |
| Peanut oil . . . . .                    | 102.4                        | 98.4                         |
| Crisco . . . . .                        | 78.8                         | 81.8                         |
| Lard . . . . .                          | 63.2                         | 71.7                         |
| Butter fat . . . . .                    | 35.8                         | 55.5                         |
| Coconut oil . . . . .                   | 7.7                          | 35.3                         |

the body fat formed is clearly marked; but also there may here be seen the tendency of the body to modify the fat absorbed, so that the iodine numbers of the different body fats do not differ *so greatly* as do those of the corresponding food fats.

### Lipids as Body Constituents

From what has been stated above, fat is seen to be a form of reserve fuel to which any of the organic foodstuffs may contribute



(see also Chapter VII). It is as reserve fuel that the large deposits of body fat are chiefly significant, but it should not be forgotten that even this "depot fat" may function as a protection to the body from mechanical injury and too rapid a loss of heat when exposed to cold, and as a packing and support to the visceral organs, particularly the kidneys.

As noted above, some particular fatty acid or acids appear to be nutritionally essential in the sense that they are indispensable to the body and must be supplied to it by the food.

The phospholipids and the sterols are both essential to the normal structure and functioning of the body.

Thus cell membranes are not simply walls of protein matter but are composed of both proteins and lipids of different kinds and in varying proportions, and the organic matter of protoplasm is to be regarded as regularly containing lipids as well as proteins.

Bloor states that "there is always a large proportion of the fatty materials of tissues which cannot be seen with the microscope, cannot be stained by the usual fat stains and cannot be extracted by the ordinary fat solvents such as ether, chloroform, or benzene; but may be removed, although much of it with difficulty, by alcohol either cold or hot. The nature of this lipid material and its relationship to the fat of food and (body) stores constitutes one of the important problems in the field of fat metabolism at the present time."

As indicated in the classification quoted from Bloor above, the phospholipids (also called phospholipins and phosphatids) are "substituted fats" containing nitrogen and phosphorus. The familiar subdivision is into lecithin(s), cephalin(s), and sphingomyelin(s). Bloor makes a coordinate group of the glycolipids (cerebrosides, galactolipins).

The Macleans use the following classification for the members of these two groups:

- I. Phospholipins (phospholipids, phosphatids)
  - A. Monoaminomonophospholipins (atomic ratio, N:P::1:1)
    - (a) Lecithins; (b) Cephalins (kephalins).
  - B. Diaminomonophospholipins (N:P::2:1)
    - Sphingomyelins.
- II. Galactolipins (glycolipids, cerebrosides)
  - (a) Phrenosin; (b) Kerasin; (c) Nervon.

The chief chemical relationships are as follows:

Lecithin has the molecular structure of a fat in which one of the fatty acid radicles is replaced by a substituted phosphoric acid which carries a choline radicle.

Cephalin has the same general structure but its nitrogenous radicle is different.

Sphingomyelin is not a glyceride (contains no glyceryl radicle and so is not a substituted fat), but is related to the fats, lecithins, and cephalins in that it contains a fatty acid radicle and a choline-phosphoric acid radicle. • Each of these is linked to a central sphingosine radicle.

The cerebrosides (galactolipins, glycolipids) do not contain phosphorus but do contain fatty acid and nitrogen, the latter in the form of a sphingosine group which is thus a common feature of the sphingomyelin and cerebroside molecules.

Details of structure can readily be found in the textbooks, monographs, and reviews listed at the end of the chapter.

**Phospholipids** or **phosphatids** are widely distributed in living cells and doubtless essential to their structure and functions. The fatty matter (total lipids) of the active tissues of the body, as distinguished from that of the adipose tissue, seems to consist largely of phospholipids.

In all of the cases studied structurally by Levene, the lecithin molecule contains at least one unsaturated fatty acid; and Bloor and his coworkers (among others) emphasize the fact that in general there is a larger proportion of unsaturation in the fatty acids of the phosphatids than in those of the "neutral" or "depot" fats (the triglycerides such as those of the adipose tissue). It is largely for this reason that the phosphatids are regarded as possible intermediary steps in the metabolism of fatty acids. Whether or not they function thus in fat metabolism, the phospholipids are undoubtedly essential constituents of cell structure.

**Sterols** also are widely distributed in plant and animal cells and tissues. Doubtless many sterols are essential constituents of the organisms in which they occur. Some but not all sterols are among the vitamins D (Chapter XXIII).

**Other lipoidal substances** such as bile acids, sex hormones, and such things of primarily pathological interest as the carcinogenic hydrocarbons cannot be included within the scope of this book. Nor is space available here for the consideration of lipoids from

the viewpoint of their occurrence in vegetable tissues and their significance in the chemical processes of plant life.

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## CHAPTER IV

### GENERAL CHEMISTRY OF THE PROTEINS AND THEIR AMINO ACIDS

CARBOHYDRATES and fats are the chief sources of energy for the activities of the body but not the chief constituents of which the active tissues are composed. Muscle tissue, for instance, contains but little carbohydrate, and often very little fat. The chief organic constituents of the muscles, and of protoplasm generally, are substances which contain nitrogen and sulfur in addition to carbon, hydrogen, and oxygen. Mulder, in 1838, described a nitrogenous material which he believed to be the fundamental constituent of tissue substances and gave it the name *protein*, derived from a Greek verb meaning "to take the first place." While Mulder's chemical work did not prove to be of permanent value, the term which he introduced has been retained, and in the plural form, *proteins*, is now used as a group name to cover a large number of different but related nitrogenous organic compounds which are so prominent among the constituents of the tissues and of food that they may still be accorded some degree of preëminence in a study of the chemistry of food and nutrition.

Proteins are essential constituents of both plant and animal cells. There is no known life without them. Plants build their own proteins from inorganic materials obtained from the soil and air. Animals form the proteins characteristic of their own tissues, but in general they cannot build them up from simple inorganic substances such as suffice for the plants, and must depend upon the digestion products obtained from the proteins of their food. Since animals must have proteins for the construction and repair or maintenance of their tissues, and since, broadly speaking, they cannot make their proteins except from the cleavage products of other proteins, it follows that proteins (or their cleavage products, the amino acids) are necessary ingredients of the food of all animals.

## Chemical Nature and Physical Properties of Proteins in General

Generally speaking, the proteins of different kinds of tissue, and even of the corresponding tissues of different species, are not identical substances. The total number of different proteins occurring in nature must therefore be very great. Such natural proteins as have been sufficiently isolated and studied as to warrant description as chemical individuals have proven to be very complex substances and in no case has the chemical structure of a natural protein been fully determined. It has, however, been shown that the typical proteins are essentially anhydrides of the following amino acids:

### Chemical Structures of the Amino Acids

#### *Monoamino-monocarboxylic acids*

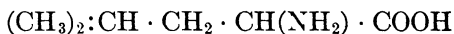
Glycine, amino-acetic acid,  $\text{CH}_2(\text{NH}_2) \cdot \text{COOH}$

Alanine,  $\alpha$ -amino-propionic acid,  $\text{CH}_3 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$

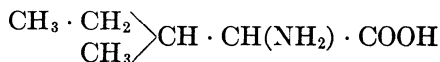
Valine,  $\alpha$ -amino- $\beta$ -methyl-*n*-butyric acid,



Leucine,  $\alpha$ -amino- $\gamma$ -methyl-*n*-valeric acid,



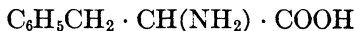
Isoleucine,  $\alpha$ -amino- $\beta$ -methyl-*n*-valeric acid,



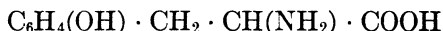
Norleucine,  $\alpha$ -amino-*n*-caproic acid,



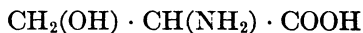
Phenylalanine,  $\alpha$ -amino- $\beta$ -phenyl-propionic acid,



Tyrosine,  $\alpha$ -amino- $\beta$ -para-hydroxyphenyl-propionic acid,



Serine,  $\alpha$ -amino- $\beta$ -hydroxy-propionic acid,



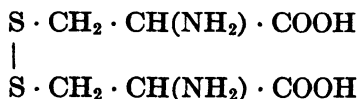
Threonine,\*  $\alpha$ -amino- $\beta$ -hydroxy-*n*-butyric acid,



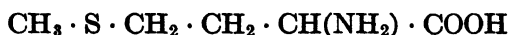
\* To W. C. Rose and coworkers we are indebted for the discovery of this hitherto unknown substance, its actual (physical) isolation, its chemical identification, and the name indicative of its structural relationship to the tetrose sugar, threose.



Cystine (dicysteine), or di-( $\alpha$ -amino- $\beta$ -thio-propionic acid),



Methionine,  $\alpha$ -amino- $\gamma$ -methylthio-*n*-butyric acid,

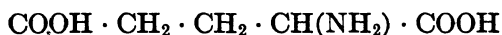


*Monoamino-dicarboxylic acids*

Aspartic acid, amino-succinic acid,



Glutamic (glutaminic) acid,  $\alpha$ -amino-glutaric acid,

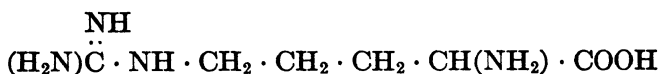


Hydroxyglutamic acid,  $\alpha$ -amino- $\beta$ -hydroxy-glutaric acid,

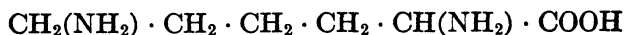


*Diamino-monocarboxylic acids*

Arginine,  $\alpha$ -amino- $\delta$ -guanidino-*n*-valeric acid,

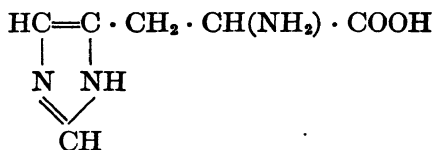


Lysine,  $\alpha$ ,  $\epsilon$ , diamino-*n*-caproic acid,

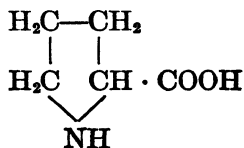


*Heterocyclic amino acids*

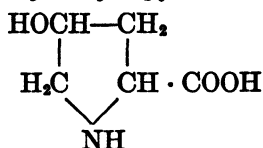
Histidine,  $\alpha$ -amino- $\beta$ -imidazole propionic acid,



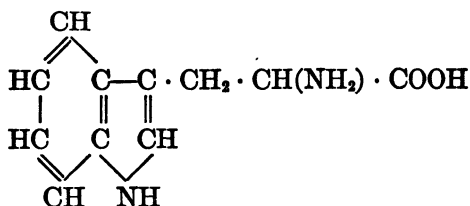
Proline,  $\alpha$ -pyrrolidine-carboxylic acid,



Hydroxyproline,  $\gamma$ -hydroxy- $\alpha$ -pyrrolidine-carboxylic acid,



Tryptophane,  $\alpha$ -amino- $\beta$ -indole-propionic acid,



For convenience of reference there are given in Table 2, on page 46, for the chief amino acids which make up natural proteins: (1) the directions in which the naturally occurring forms rotate the plane of polarized light; and (2) elementary compositions which will be interesting to compare with those of typical proteins as shown in Table 4.

In addition to these amino acids which occur in proteins generally, there are several which, so far as known, belong only to a single protein or small group of proteins. Recently the observation has been made, that, besides amino acids most (if not all) proteins also contain a carbohydrate radicle which persists through extensive purifications and is regarded as a component of the molecule. From certain proteins a carbohydrate complex consisting of two molecules of hexose and one molecule of hexosamine has been isolated. In a number of proteins, the hexose present has been identified as mannose, galactose, or a mixture of mannose and galactose. Further discussion of the carbohydrate radicles of proteins is given by Rimington (1936).

It will be noted that the amino-acid constituents of the protein molecule differ much in structure among themselves. They are, however, all  $\alpha$ -amino acids, *i.e.*, the amino group (or one of them if there be more than one) is attached to the carbon atom adjacent to the carboxyl. Proline and hydroxyproline may be regarded as only apparently exceptions to this statement, as in them the  $\alpha$ -amino group is "condensed" with the  $\delta$ -carbon.

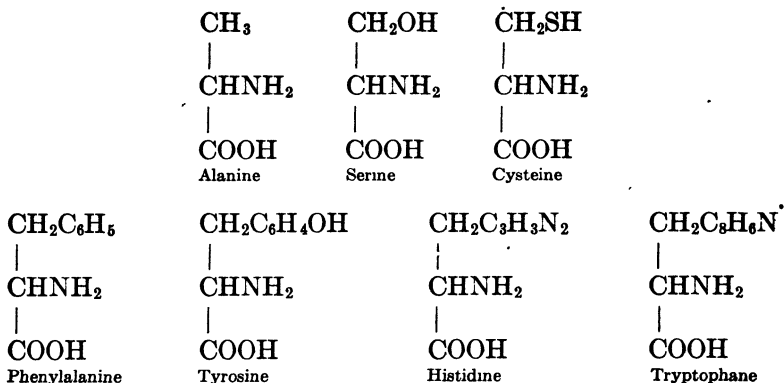
In view of the wide occurrence of the alanine radicle in proteins

TABLE 2. — OPTICAL ACTIVITY<sup>1</sup> AND ELEMENTARY COMPOSITION OF NATURAL AMINO ACIDS

| NAME                                     | CARBON          | HYDROGEN        | OXYGEN          | NITROGEN        | SULFUR          |
|--|-----------------|-----------------|-----------------|-----------------|-----------------|
|  | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| Glycine . . . . .                        | 31.98           | 6.71            | 42.64           | 18.67           | —               |
| <i>d</i> -Alanine . . . . .              | 40.42           | 7.93            | 35.92           | 15.73           | —               |
| <i>l</i> -Serine . . . . .               | 34.26           | 6.72            | 45.68           | 13.34           | —               |
| <i>d</i> -Valine . . . . .               | 51.24           | 9.47            | 27.33           | 11.96           | —               |
| <i>l</i> -Leucine . . . . .              | 54.97           | 9.99            | 24.35           | 10.69           | —               |
| <i>d</i> -Isoleucine . . . . .           | 54.97           | 9.99            | 24.35           | 10.69           | —               |
| <i>d</i> -Norleucine . . . . .           | 54.97           | 9.99            | 24.35           | 10.69           | —               |
| <i>l</i> -Aspartic acid . . . . .        | 36.08           | 5.31            | 48.11           | 10.53           | —               |
| <i>d</i> -Glutamic acid . . . . .        | 40.80           | 6.17            | 43.51           | 9.50            | —               |
| <i>l</i> -Hydroxyglutamic acid . . . . . | 36.79           | 5.52            | 49.06           | 8.63            | —               |
| <i>l</i> -Cystine . . . . .              | 29.97           | 5.03            | 26.66           | 11.65           | 26.69           |
| <i>l</i> -Threonine . . . . .            | 40.31           | 7.62            | 40.31           | 11.76           | —               |
| <i>l</i> -Methionine . . . . .           | 40.23           | 7.43            | 21.45           | 9.39            | 21.50           |
| <i>d</i> -Arginine . . . . .             | 41.34           | 8.10            | 18.38           | 32.18           | —               |
| <i>d</i> -Lysine . . . . .               | 49.27           | 9.66            | 21.90           | 19.17           | —               |
| <i>l</i> -Phenylalanine . . . . .        | 65.41           | 6.72            | 19.38           | 8.49            | —               |
| <i>l</i> -Tyrosine . . . . .             | 59.64           | 6.12            | 26.50           | 7.74            | —               |
| <i>l</i> -Histidine . . . . .            | 46.42           | 5.85            | 20.63           | 27.10           | —               |
| <i>l</i> -Tryptophane . . . . .          | 64.67           | 5.93            | 15.68           | 13.72           | —               |
| <i>l</i> -Proline . . . . .              | 52.14           | 7.88            | 27.81           | 12.17           | —               |
| <i>l</i> -Hydroxyproline . . . . .       | 46.77           | 6.92            | 36.62           | 10.69           | —               |

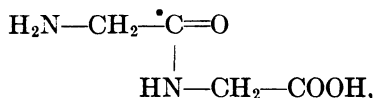
<sup>1</sup> It has been customary to denote the naturally occurring form of the various amino acids by *d*- or *l*-, accordingly as they are dextro- or levo-rotatory. However, recent studies have revealed the interesting fact that all of the amino acids which occur in proteins are stereochemically related, that is, there is an identical spatial arrangement of the groups about the alpha carbon atom in the natural form of the different amino acids. For this reason, there is an increasing tendency among some investigators to designate as the *l*-form that form of the amino acid which occurs in nature; to indicate the optical activity, a *plus* or *minus* sign is employed. For example, the new designation of naturally occurring alanine and serine would be *l*-(+)-alanine and *l*-(-)-serine, instead of the formerly used *d*-alanine and *l*-serine, respectively.

and the fact that we shall have occasion to discuss the behavior of alanine (as a typical amino acid) in metabolism, it may be of interest to point out that several of the amino acids, even including some of unique constitution, may be regarded as derived from alanine by the substitution of a simple or complex radicle for one of the hydrogens on the  $\beta$  carbon of alanine. Thus by the substitution of an OH or SH group one obtains serine or cysteine respectively; by substituting the phenyl or hydroxyphenyl group, there results phenylalanine or tyrosine; by the imidazole ( $C_3H_3N_2$ ),<sup>\*</sup> histidine; by the indole ( $C_8H_6N$ ) radicle, tryptophane:



### Amino Acids in the Protein Molecule

The linkage of the amino acid radicles in the protein molecule is chiefly (but probably not exclusively) through the carboxyl group of one amino acid reacting with the amino group of another. Thus two molecules of glycine combined by elimination of one molecule of water yield glycylglycine,



which is the simplest of an immense group of anhydrides of amino acids, all of which are called *peptides*. Dipeptides contain two amino acid radicles, tripeptides contain three, etc.

A certain analogy between the chief carbohydrates and proteins of the food may be noted. As starch on hydrolysis yields the polysaccharide dextrins, the disaccharide maltose, and finally (as end product) the monosaccharide glucose, so a typical native protein is hydrolyzed through proteoses, peptones, polypeptides, and di- or tri-peptides, to amino acids. Thus the amino acid bears the same general relation to the protein which glucose bears to starch; and just as the molecular weight of starch is very high and a single starch molecule yields a large number of monosaccharide molecules, so the molecular weight of the typical protein is very high and the protein molecule yields a large number of amino acid molecules. There is, however, this important difference: the molecules of monosaccharide resulting from complete hydrolysis

of starch are all alike (glucose), whereas the complete hydrolysis of any typical protein yields several of the above-mentioned kinds of amino acids, in the case of most proteins from twelve to twenty.

In view of the marked differences in chemical structure and nutritive function existing among these amino acids, it becomes important to know the relative proportions in which the various amino acid radicles exist in the different proteins. This is studied by hydrolyzing the protein and separating and recovering as completely as possible the amino acids resulting from the hydrolysis. Since the recovery of most of the amino acids cannot be accomplished without loss by the methods in use at present, the results obtained are not strictly quantitative and our knowledge of the radicles which make up the protein molecule remains incomplete.

Table 3 shows the percentages of amino acids obtained from

TABLE 3. — PERCENTAGES OF INDIVIDUAL AMINO ACIDS FOUND IN FOUR DIFFERENT PROTEINS

|                                  | CASEIN | GELATIN | GLIADIN | ZEIN               |
|----------------------------------|--------|---------|---------|--------------------|
| Glycine . . . . .                | 0.50   | 25.5    | 0.46    | 0.0                |
| Alanine . . . . .                | 1.85   | 8.7     | 2.0     | 9.8                |
| Valine . . . . .                 | 6.7    | 1.0     | 3.3     | 1.9                |
| "Leucine" <sup>a</sup> . . . . . | 9.7    | 7.1     | 6.6     | 25.0               |
| Proline . . . . .                | 8.0    | 9.5     | 13.2    | 9.0                |
| Hydroxyproline . . . . .         | 0.23   | 14.1    | ?       | ?                  |
| Phenylalanine . . . . .          | 3.9    | 1.4     | 2.4     | 7.6                |
| Glutamic acid . . . . .          | 21.8   | 5.8     | 43.7    | 31.3               |
| Hydroxyglutamic acid . . . . .   | 10.5   | 0.0     | 2.4     | 2.5                |
| Aspartic acid . . . . .          | 4.1    | 3.4     | 0.80    | 1.8                |
| Serine . . . . .                 | 0.50   | 0.4     | 0.13    | 1.0                |
| Tyrosine . . . . .               | 6.5    | 0.01    | 3.5     | 5.9                |
| Cystine . . . . .                | 0.34   | 0.31    | 2.1     | 1.0                |
| Histidine . . . . .              | 1.83   | 0.9     | 3.4     | 0.8                |
| Arginine . . . . .               | 3.8    | 8.2     | 3.1     | 1.8                |
| Lysine . . . . .                 | 6.3    | 5.9     | 0.92    | 0.0                |
| Tryptophane . . . . .            | 2.2    | 0.0     | 1.14    | 0.0                |
| Ammonia . . . . .                | 1.61   | 0.4     | 5.2     | 3.6                |
| Summation <sup>b</sup> . . . . . | 90.36  | 92.62   | 94.35   | 103.0 <sup>b</sup> |

<sup>a</sup> Includes leucine and isoleucine; also norleucine if present.

<sup>b</sup> The actual total weight of the products of complete hydrolysis is obviously greater than the weight of the original protein.

four proteins chosen as typical of those occurring in different food materials, while corresponding (but in many cases less complete) data for a larger number of proteins are given in Table 5 beyond.

It is evident that these tables do not contain data for the more recently discovered amino acids, methionine and threonine, which are, however, recognized to occur widely; nor for the sugar component of proteins. Often, too, the best determination of an amino acid is too low because of losses in its separation. The recognition of this incompleteness of the data for the *known* protein constituents makes it unnecessary to assume (in order to account for the portion of the original protein not yet recovered) that these proteins contain any large amount of other amino acids as yet unidentified. On the other hand, it is well to remember that there are hints that some proteins at least may conceivably contain building stones which have not yet been recognized.

From the data given in Table 3 it will be seen that the proportions in which a given amino acid radicle occurs in various proteins may be quite different. The four proteins here shown yield from 0.0 to 25.5 per cent of glycine; from 1.85 to 9.8 per cent of alanine; from 1.0 to 6.7 per cent of valine; from 6.6 to 25.0 per cent of leucine. Of lysine, zein yields none, gliadin about 1 per cent, gelatin and casein about 6 per cent. Of tryptophane, zein and gelatin yield none, gliadin about 1 per cent, casein about 2 per cent.

For more detailed comparisons of the percentages of amino acids in different proteins and also in the flesh of four widely separated species, the more extended Table 5 further on in this chapter may be consulted.

*The ultimate composition* of the proteins shows a general similarity throughout the group. All contain carbon, hydrogen, oxygen, nitrogen, and sulfur; some also phosphorus or iron.

From the accompanying table of ultimate composition of twelve typical proteins, it will be seen that all these proteins contain 51 to 55 per cent carbon, about 7 per cent hydrogen, 20 to 23 per cent oxygen, 15.5 to 18.7 per cent nitrogen, 0.3 to 2.0 per cent sulfur. Other typical proteins thus far studied have shown ultimate composition within these same limits.

Similarity of elementary composition is entirely consistent with the belief that there may be an enormous number of chemical individuals among the proteins of nature.

TABLE 4. — ULTIMATE (ELEMENTARY) COMPOSITION OF SOME TYPICAL PROTEINS ACCORDING TO OSBORNE

|                      | CARBON          | HYDRO-<br>GEN   | NITRO-<br>GEN   | OXYGEN          | SULFUR          | IRON            | PHOS-<br>PHORUS |
|----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                      | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| Egg albumin . . .    | 52.75           | 7.10            | 15.51           | 23.024          | 1.616           | —               | —               |
| Lactalbumin . . .    | 52.19           | 7.18            | 15.77           | 23.13           | 1.73            | —               | —               |
| Leucosin . . .       | 53.02           | 6.84            | 16.80           | 22.06           | 1.28            | —               | —               |
| Serum-globulin . . . | 52.71           | 7.01            | 15.85           | 23.32           | 1.11            | —               | —               |
| Myosin . . .         | 52.82           | 7.11            | 16.67           | 22.03           | 1.27            | —               | —               |
| Edestin . . .        | 51.50           | 7.02            | 16.69           | 21.91           | 0.88            | —               | —               |
| Legumin . . .        | 51.72           | 6.95            | 18.04           | 22.905          | 0.385           | —               | —               |
| Casein . . .         | 53.13           | 7.06            | 15.78           | 22.37           | 0.80            | —               | 0.86            |
| Ovovitellin . . .    | 51.56           | 7.12            | 16.23           | 23.242          | 1.028           | —               | 0.82            |
| Gliadin . . .        | 52.72           | 6.86            | 17.66           | 21.733          | 1.027           | —               | —               |
| Zein . . .           | 55.23           | 7.26            | 16.13           | 20.78           | 0.60            | —               | —               |
| Oxyhemoglobin . . .  | 54.64           | 7.09            | 17.38           | 20.165          | 0.39            | 0.335           | —               |

Fischer illustrated the vast number of isomers which may exist among polypeptides and proteins by pointing out that a synthetic 19-peptide obtained by linking 15 glycine and 4 leucine molecules is only one of 3876 possible isomers, without considering the tautomerism of the peptide linking. When more than two kinds of amino acids are involved, the possible number of isomers increases very rapidly. If a protein be imagined made up of 30 molecules of 18 different amino acids, one taken twice, one 3 times, another 3, one 4, one 5 times, and 13 taken once each, there would be  $10^{27}$  isomers even if there were no tautomerism of the peptide group and if the linking took place only in the simple way as with mono-amino-monocarboxylic acids.

It is easy to see that when one considers not only isomerism but the vast number of compounds of slightly different composition which can be obtained by varying the kinds and proportions of the amino acid radicles in the protein molecule, the possible number of different proteins of very similar elementary composition is practically unlimited.

### Probable Molecular Weights of Proteins

The minimum molecular weight which would be consistent with the results of ultimate analysis would for many proteins be about 16,000 to 18,000. Thus, oxyhemoglobin contains only 0.335 per cent of iron, and since there must be at least one iron atom in the molecule, it is obvious from a simple proportion making use of the atomic weight of iron,

$$0.335:56::100:x,$$

that the molecular weight of hemoglobin must be in the neighborhood of 16,700 or a multiple of this.

Physico-chemical studies have now made it evident that the probable molecular weights of typical proteins are of the order of the multiples rather than of the analytically conceivable minimum just calculated. Thus, Cohn, Sørensen, and Svedberg, working independently and with different physico-chemical methods, all obtained data indicating for egg albumin a molecular weight of about 34,500. Svedberg, using methods for the direct determination of molecular weights, based upon the behavior of protein "solutions" (molecular dispersions) in an ultracentrifuge, found that the molecular weights of many typical proteins were approximately 1, 2, 3, or 6 times the molecular weight of egg albumin, while even much higher molecular weights were indicated in some cases.

The student will do well at this point to read carefully (whether as review or extension of the background which he brings to the present study) the chapters on proteins and amino acids in such modern textbooks as Conant's *Chemistry of Organic Compounds* and Thomas' *Colloid Chemistry*.

### Further Chemical Properties of Proteins

In some cases proteins have been obtained in crystalline form. Generally speaking the proteins may be regarded as typically colloidal substances. In view of the fact that the behavior of proteins in the tissues is largely dependent upon their colloidal character it is of interest to bear in mind the very high molecular weights of the proteins as mentioned in the last paragraph. These enormously high molecular weights make it possible that in the case of a typical native protein the individual molecule is so large as to constitute a colloidal particle so that here the distinction between molecular and colloidal dispersions may disappear.

The amino acids of which the proteins are composed are, by virtue of their amino and carboxyl groups, amphoteric substances or ampholytes, capable of dissociating either as cations or as anions, depending upon the reaction (hydrogen ion activity) of the solution. For discussion of amphoteric nature in terms of the *zwitter-ion* theory see Thomas' *Colloid Chemistry*, Cohn's (1939) review, and other readings suggested at the end of this chapter.

The proteins also are amphoteric substances. As the protein molecules may dissociate either as cations or as anions, depending upon the hydrogen ion activity of the solution, it is evident that



there must be some hydrogen ion activity at which the tendencies toward the acidic and basic dissociations are equal. This is known as the *iso-electric point* because at this reaction the protein does not migrate in an electric field. This point or zone has been established for many proteins and other ampholytes. At this hydrogen ion activity, proteins are most easily precipitated by neutral salts or alcohol. This fact has been extensively used in work dealing with the purification of proteins and other amphoteric substances and in attempts to gain more knowledge of their chemical nature.

The proteins are insoluble in all of the usual solvents for fats (ether, acetone, chloroform, carbon disulfide, carbon tetrachloride, benzene, and petroleum distillate). They differ in their solubilities in water, salt solutions, and alcohol, and these differences play a considerable part in the present schemes of classification.

### Classification

There was formerly considerable confusion in the classification and terminology of the proteins, and some differences of usage will still be met in the literature. At present, however, the majority of writers follow the recommendations made by a joint committee of the American Physiological Society and the American Society of Biological Chemists in 1907. The following is an outline of the classification thus recommended, to which have been added examples from among the food proteins.

I. SIMPLE PROTEINS. Protein substances which yield only amino acids or their derivatives on hydrolysis.\*

(a) *Albumins*. Simple proteins soluble in pure water and coagulable by heat. Examples: egg albumin, serum albumin (blood), leucosin (wheat), legumelin (peas).

(b) *Globulins*. Simple proteins insoluble in pure water, but soluble in neutral salt solutions. Examples: muscle globulin, serum globulin (blood), edestin (wheat, hemp seed, and other seeds), phaseolin (beans), legumin (beans and peas), vignin (cow peas), tuberin (potato), amandin (almonds), excelsin (Brazil nuts), arachin and conarachin (peanuts).

(c) *Glutelins*. Simple proteins insoluble in all neutral solvents, but readily soluble in very dilute acids and alkalies. The best-

\* This definition is not literally correct, since recent studies have shown the presence of carbohydrate material in many of these so-called simple proteins.

known and most important member of this group is the glutenin of wheat.

(d) *Alcohol soluble proteins (prolamins)*. Simple proteins soluble in relatively strong alcohol (70–80 per cent) but insoluble in water, absolute alcohol, and other neutral solvents. Examples: gliadin (wheat), zein (maize), hordein (barley), kafirin (kafir corn).

(e) *Albuminoids*. These are the simple proteins characteristic of the skeletal structures of animals (for which reason they are also called scleroproteins) and also of the external protective tissues, such as the skin, hair, etc. Example: collagen, which when boiled with water yields gelatin.

(f) *Histones*. Soluble in water, and insoluble in very dilute ammonia, and in the absence of ammonium salts insoluble even in an excess of ammonia; yield precipitates with solutions of other proteins and a coagulum on heating which is easily soluble in very dilute acids. On hydrolysis they yield several amino acids, among which the basic ones predominate. The only members of this group which have any considerable importance as food are the thymus histone and the globin of hemoglobin.

(g) *Protamins*. These are simpler substances than the preceding groups, are soluble in water, not coagulable by heat, possess strong basic properties, and on hydrolysis yield a few amino acids among which the basic amino acids greatly predominate. They are of no importance as food.

II. CONJUGATED PROTEINS. Substances which contain the protein molecule united to some other molecule or molecules otherwise than as a salt.

(a) *Nucleoproteins*. Compounds of one or more protein molecules with nucleic acid. Examples of the nucleic acids thus found united with proteins are thymo-nucleic acid (thymus gland), tritico-nucleic acid (wheat germ).

(b) *Glycoproteins*. Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid. Example: mucins.

(c) *Phosphoproteins*. Compounds in which the phosphorus is in organic union with the protein molecule otherwise than in a nucleic acid or lecithin. Examples: caseinogen (milk), ovovitellin (egg yolk).

(d) *Hemoglobins*. Compounds of the protein molecule with hematin or some similar substance. Example: hemoglobin of blood. (The redness of meat is also due chiefly to hemoglobin).

(e) *Lecithoproteins*. Compounds of the protein molecule with lecithins or related substances.

### III. DERIVED PROTEINS.

1. *Primary protein derivatives*. Derivatives of the protein molecule apparently formed through hydrolytic changes which involve only slight alterations.

(a) *Proteans*. Insoluble products which apparently result from the incipient action of water, very dilute acids, or enzymes. Examples: casein (curdled milk), fibrin (coagulated blood).

(b) *Metaproteins*. Products of the further action of acids and alkalies whereby the molecule is sufficiently altered to form proteins soluble in very weak acids and alkalies, but insoluble in neutral solvents. This group includes the substances which have been called "acid proteins," "acid albumins," "syntonin," "alkali proteins," "alkali albumins," and "albuminates."

(c) *Coagulated proteins*. Insoluble products which result from (1) the action of heat on protein solutions, or (2) the action of alcohol on the protein. Example: cooked egg albumin, or egg albumin precipitated by means of alcohol.

2. *Secondary protein derivatives*. Products of the further hydrolytic cleavage of the protein molecule.

(a) *Proteoses*. Soluble in water, not coagulable by heat, precipitated by saturating their solutions with ammonium sulfate or zinc sulfate. The products commercially known as "peptones" consist largely of proteoses.

(b) *Peptones*. Soluble in water, not coagulable by heat, and not precipitated by saturating their solutions with ammonium sulfate or zinc sulfate. These represent a further stage of cleavage than the proteoses. (The term "peptone" was formerly applied to all digestion products not coagulated by boiling, and is still popularly used in the same sense.)

(c) *Peptides*. Definitely characterized combinations of two or more amino acids. An anhydride of two amino acid radicles is called a "di-peptide"; one having three amino acid radicles, a "tri-peptide"; etc. Peptides result from the further hydrolytic cleavage of the peptones. As was mentioned above, many peptides have also been made in the laboratory by the linking together of amino acids.

Substances simpler than the peptones but containing several amino acid radicles are often called "poly-peptides."

### Quantitative Studies of the Amino Acid Make-up of Proteins

In recent years there has been a rapid simultaneous development of knowledge of the differing values or efficiencies of individual proteins in nutrition and their differences in chemical structure as shown in the relative proportions of the different amino acids which they yield on hydrolysis. Illustrative data of the latter sort are shown in Table 3 and a compilation of the amino-acid make-up of a larger number of proteins in Table 5.

Attempts to determine the percentage of each amino acid obtainable from a given protein are so costly and time-consuming, and require such large quantities of the purified protein material, that data of this sort can be accumulated only very slowly. If this were the only means of obtaining an insight into the amino-acid make-up of proteins, progress in this direction would often be indefinitely delayed. Van Slyke has, however, devised a method of fractioning the nitrogen of a protein into eight parts, four of which are measures of individual amino acids.

In order to accomplish this, the protein is hydrolyzed, the basic amino acids precipitated by means of phosphotungstic acid, and this precipitate fractionated to show how much of its nitrogen exists in each of the four forms, arginine, histidine, lysine, and cystine. The nitrogen of the filtrate is fractionated into amino and non-amino nitrogen. The two other forms of nitrogen determined are the amide nitrogen of the protein which appears as ammonia in the hydrolysis, and the humin or melanin nitrogen which is the nitrogen found in the humus-like insoluble material formed during the hydrolysis of the protein. The amino nitrogen of the filtrate corresponds to that of the glycine, alanine, valine, leucine, isoleucine, serine, phenylalanine, tyrosine, aspartic acid, glutamic and hydroxyglutamic acid, and one half of the tryptophane which remains after the hydrolysis of the protein. The other half of the nitrogen of tryptophane appears with the nitrogen of proline and hydroxyproline in the non-amino nitrogen fraction of the filtrate. The results of a Van Slyke analysis of a protein are expressed, not in terms of percentage of amino acid or acids, but as the percentage distribution of the total nitrogen of the protein among the eight fractions just mentioned. The results of applying this method to some typical proteins are shown in Table 6.

As the Van Slyke method consists in fractioning the total

TABLE 5. — PERCENTAGES OF AMINO ACIDS FROM HYDROLYSIS OF VARIOUS PROTEINS

|                               | ALBUMINS         |             |           |          | GLOBULINS            |                     |                        |                          |                        |                   |                          |                   |                     | GLUTELINS           |                   |
|-------------------------------|------------------|-------------|-----------|----------|----------------------|---------------------|------------------------|--------------------------|------------------------|-------------------|--------------------------|-------------------|---------------------|---------------------|-------------------|
|                               | Egg Albumin      | Lactalbumin | Legumelin | Leucosin | Amandin<br>(Almonds) | Coconut<br>Globulin | Edestin<br>(Hemp Seed) | Excelsin<br>(Brazil Nut) | Glycinin<br>(Soy Bean) | Legumin<br>(Peas) | Phaseolin<br>(Navy Bean) | Vicilin<br>(Peas) | Vignin<br>(Cow Pea) | Glutenin<br>(Wheat) | Maize<br>Glutelin |
| Glycine . . .                 | 1.75             | 0.37        | 0.50      | 0.94     | 0.51                 | trace               | 3.8                    | 0.60                     | 1.54                   | 0.38              | 0.55                     | 0.00              | 0.00                | 0.89                | 0.75              |
| Alanine . . .                 | 8.1              | 2.4         | 0.92      | 4.4      | 1.40                 | 4.1                 | 3.6                    | 2.3                      | "                      | 2.1               | 1.80                     | 0.50              | 0.97                | 6.16                | "                 |
| Valine . . .                  | 2.5              | 3.3         | 0.69      | 0.18     | 0.16                 | 3.6                 | 6.2                    | 1.51                     | 0.68                   | "                 | 1.04                     | 0.15              | 0.34                | 1.02                | "                 |
| "Leucine" . .                 | 10.7             | 14.0        | 9.6       | 11.3     | 4.4                  | 6.0                 | 14.5                   | 8.7                      | 8.4                    | 8.0               | 9.6                      | 9.4               | 7.8                 | 6.3                 | 6.2               |
| Proline . . .                 | 3.6              | 3.8         | 4.0       | 3.2      | 2.4                  | 5.5                 | 4.1                    | 3.6                      | 3.8                    | 3.2               | 2.8                      | 4.1               | 5.2                 | 6.15                | 5.0               |
| Hydroxyproline .              | "                | "           | "         | "        | "                    | "                   | "                      | "                        | "                      | "                 | "                        | "                 | "                   | "                   | "                 |
| Phenylalanine .               | 5.32             | 1.25        | 4.8       | 3.8      | 2.5                  | 2.0                 | 3.1                    | 3.6                      | 3.9                    | 3.8               | 3.2                      | 3.8               | 5.3                 | 2.75                | 1.74              |
| Aspartic acid .               | 6.2              | 9.3         | 4.1       | 3.4      | 5.4                  | 5.1                 | 10.2                   | 3.8                      | 9.4                    | 5.3               | 5.2                      | 5.3               | 4.0                 | 2.03                | 0.63              |
| Glutamic acid .               | 13.3             | 12.9        | 13.0      | 6.7      | 23.1                 | 19.1                | 19.2                   | 12.9                     | 19.5                   | 17.0              | 14.5                     | 21.3              | 16.9                | 26.49               | 12.7              |
| Hydroxyglutamic<br>acid . . . | "                | 10.0        | "         | "        | "                    | "                   | "                      | "                        | "                      | "                 | "                        | "                 | "                   | 1.80                | "                 |
| Serine . . .                  | "                | 1.76        | "         | "        | "                    | 1.76                | 0.33                   | "                        | "                      | 0.53              | 0.38                     | "                 | "                   | 0.74                | "                 |
| Tyrosine . . .                | 1.77             | 1.95        | 1.56      | 3.3      | 1.12                 | 3.2                 | 4.6                    | 3.0                      | 1.86                   | 1.55              | 2.8                      | 2.4               | 2.3                 | 5.35                | 5.0               |
| Cystine . . .                 | 1.21             | 2.56        | "         | "        | 0.85                 | 1.54                | 1.75                   | 1.84                     | 1.27                   | 0.90              | 0.16                     | 0.57              | 0.52                | 1.80                | 0.52              |
| Arginine . . .                | 4.9              | 3.5         | 5.4       | 5.9      | 11.8                 | 15.9                | 15.8                   | 16.1                     | 8.1                    | 11.7              | 4.9                      | 8.9               | 7.2                 | 4.72                | 7.6               |
| Histidine . . .               | 1.71             | 2.6         | 2.3       | 2.8      | 1.58                 | 2.4                 | 4.0                    | 2.5                      | 2.1                    | 1.69              | 2.6                      | 2.5               | 3.1                 | 1.76                | 3.0               |
| Lysine . . .                  | 3.8              | 9.9         | 3.0       | 2.8      | 0.70                 | 5.8                 | 3.8                    | 1.64                     | 9.1                    | 5.0               | 4.6                      | 5.4               | 4.3                 | 1.92                | 6.7               |
| Tryptophane .                 | 3.6 <sup>b</sup> | 3.0         | present   | present  | 1.37                 | 1.25                | 2.5                    | 2.6                      | 1.66                   | 1.76              | 0.94                     | 0.15              | 1.65                | 2.13                | 2.1               |
| Ammonia . . .                 | 1.34             | 1.31        | 1.26      | 1.41     | 3.7                  | 1.57                | 2.3                    | 1.80                     | 2.3                    | 2.0               | 2.1                      | 2.0               | 2.3                 | 4.01                | 2.2               |
| Summation . .                 | 69.80            | 83.94       | 51.13     | 50.13    | 60.99                | 78.82               | 99.78                  | 66.49                    | 73.61                  | 64.91             | 57.17                    | 66.47             | 61.89               | 74.84               | 54.14             |

<sup>a</sup> Not determined.    <sup>b</sup> Means of data given by Jones for ovalbumin and conalbumin of egg white.

TABLE 5. — PERCENTAGES OF AMINO ACIDS FROM HYDROLYSIS OF VARIOUS PROTEINS (Continued)

|                                    | ALCOHOL-SOLUBLE PROTEINS |                     |                    |                  | ALBUMINOID | PROTOPROTEINS          |                             | MUSCLE TISSUES (NOT SINGLE PROTEINS) |         |         |         |        |
|------------------------------------|--------------------------|---------------------|--------------------|------------------|------------|------------------------|-----------------------------|--------------------------------------|---------|---------|---------|--------|
|                                    | Gliadin<br>(Wheat)       | Hordein<br>(Barley) | Prolamin<br>of Rye | Zein<br>(Maize)  | Gelatin    | Casein<br>(Cows' Milk) | Ovovitellin<br>(Hens' Eggs) | Beef                                 | Chicken | Halibut | Scallop | Shrimp |
| Glycine . . . . .                  | 0.46                     | 0.0                 | 0.13               | 0.0              | 25.5       | 0.50                   | 0.83                        | 2.1                                  | 0.68    | 0.0     | 0.0     | •      |
| Alanine . . . . .                  | 2.0                      | 0.43                | 1.33               | 9.8              | 8.7        | 1.85                   | 0.75                        | 3.7                                  | 2.3     | •       | •       | •      |
| Valine . . . . .                   | 3.3                      | 0.13                | •                  | 1.9              | 1.0        | 6.7                    | 1.87                        | 0.81                                 | •       | 0.79    | •       | •      |
| "Leucine" . . . . .                | 6.6                      | 5.7                 | 6.3                | 25.0             | 7.1        | 9.7                    | 9.9                         | 11.6                                 | 11.2    | 10.3    | 8.8     | •      |
| Proline . . . . .                  | 13.2                     | 13.7                | 9.8                | 9.0              | •9.5       | 8.0                    | 4.2                         | 5.8                                  | 4.7     | 3.2     | 2.3     | •      |
| <sup>12</sup> Hydroxyproline . . . | •                        | •                   | •                  | ?                | 14.1       | 0.23                   | •                           | •                                    | •       | •       | •       | •      |
| Phenylalanine . . . .              | 2.4                      | 5.0                 | 2.7                | 7.6              | 1.4        | 3.9                    | 2.5                         | 3.2                                  | 3.5     | 3.0     | 4.9     | •      |
| Aspartic acid . . . .              | 0.80                     | •                   | 0.25               | 1.8              | 3.4        | 4.1                    | 2.1                         | 5.9                                  | 3.2     | 8.0     | 3.5     | 7.0    |
| Glutamic acid . . . .              | 43.7                     | 43.2                | 38.0               | 31.3             | 5.8        | 21.8                   | 13.0                        | 15.5                                 | 16.5    | 13.7    | 14.9    | 15.0   |
| Hydroxyglutamic acid .             | 2.4                      | •                   | •                  | 2.5              | 0.00       | 10.5                   | •                           | •                                    | •       | •       | •       | •      |
| Serine . . . . .                   | 0.13                     | •                   | 0.06               | 1.0              | 0.4        | 0.50                   | •                           | •                                    | •       | •       | •       | •      |
| Tyrosine . . . . .                 | 3.5                      | 1.67                | 1.19               | 5.9              | 0.01       | 6.5                    | 3.4                         | 2.2                                  | 2.2     | 2.4     | 1.95    | 4.9    |
| Cystine . . . . .                  | 2.1                      | 1.55                | •                  | 1.0              | 0.31       | 0.34                   | 0.83                        | 1.55                                 | 0.64    | 0.82    | •       | 0.89   |
| Arginine . . . . .                 | 3.1                      | 2.9                 | 2.2                | 1.8              | 8.2        | 3.8                    | 7.5                         | 7.5                                  | 6.5     | 6.3     | 7.4     | 10.2   |
| Histidine . . . . .                | 3.4                      | 2.1                 | 0.39               | 0.8              | 0.9        | 1.83                   | 1.90                        | 1.76                                 | 2.5     | 2.6     | 2.0     | 3.8    |
| Lysine . . . . .                   | 0.92                     | 1.01                | •                  | 0.0              | 5.9        | 6.3                    | 4.8                         | 7.6                                  | 7.2     | 7.4     | 5.8     | 7.6    |
| Tryptophane . . . .                | 1.14                     | 1.05                | present            | 0.0 <sup>b</sup> | 0.0        | 2.2                    | 2.4                         | 1.25                                 | present | 1.25    | present | 1.21   |
| Ammonia . . . . .                  | 5.2                      | 4.8                 | 5.1                | 3.6              | 0.4        | 1.61                   | 1.25                        | 1.07                                 | 1.67    | 1.33    | 1.08    | •      |
| Summation . . . .                  | 94.35                    | 83.24               | 67.45              | 103.0            | 92.62      | 90.36                  | 57.23                       | 91.54                                | 62.79   | 61.09   | 52.63   | •      |

• Not determined. <sup>b</sup> Folin finds 0.20 per cent colorimetrically.

nitrogen without attempting to isolate individual substances, it is not subject to the losses discussed above. The tendency is rather toward possible overestimates through assigning to known substances the whole of the nitrogen found, whereas in fact some of it may have been in unknown forms.

It has been suggested that perhaps in some cases the best approximation to the truth may be obtained by taking the mean of the data obtained (1) by the Van Slyke method and (2) by the method of actual isolation of each amino acid. Thus gliadin has yielded 0.64 per cent lysine by actual isolation but has shown 1.21 per cent when examined by the method of Van Slyke; so the mean of these figures, 0.92 per cent, is generally accepted as representing the best present knowledge as to the lysine content of gliadin. In some of the compilations included in Table 5, figures for arginine, histidine, lysine, and cystine obtained by the Van Slyke method have been used.

TABLE 6. — VAN SLYKE DISTRIBUTION OF NITROGEN IN SOME TYPICAL PROTEINS

|  | CASEIN | LACTALBUMIN | GLIADIN | EDESTIN |
|--|--------|-------------|---------|---------|
| Arginine nitrogen . . . . .  | 7.41   | 7.20        | 5.45    | 27.05   |
| Histidine nitrogen . . . . .   | 6.21   | 4.57        | 3.39    | 5.75    |
| Lysine nitrogen . . . . .  | 10.30  | 12.24       | 1.33    | 3.86    |
| Cystine nitrogen (estimated from sulfur in phosphotungstate precipitate) . . . . . | 0.20   | 1.30        | 0.80    | 1.49    |
| Amino nitrogen of the filtrate . . . . .   | 55.81  | 62.00       | 51.95   | 47.55   |
| Non-amino nitrogen of the filtrate . . . . .                                       | 7.13   | 2.00        | 10.70   | 1.70    |
| Amide nitrogen . . . . .   | 10.27  | 8.57        | 24.61   | 9.99    |
| Humin or melanin nitrogen . . . . .  | 1.28   | 2.32        | 0.58    | 1.98    |

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## CHAPTER V

### NUTRITIONAL CHEMISTRY OF THE PROTEINS AND THEIR AMINO ACIDS

EXCEPT for a few cases presenting abnormalities or particular difficulties in digestion, it is now believed that the nutritive values of the food proteins depend essentially upon the kinds of amino acid radicles which they contain and the quantitative proportion of each.

As yet, however, methods for the detection and determination of the individual amino acids are still being perfected. Present knowledge is extensive and highly significant; but it is certain that many of the quantitative data are subject to improvement in precision.

Hence at the present time we *interpret* nutritional differences among proteins essentially in terms of their amino-acid make-up; but it is not yet safe to *predict* quantitative differences in nutritive value on the sole basis of the data obtainable *in vitro*. Empirical determinations of nutritive values by means of quantitatively conducted feeding experiments are still needed, as well as qualitative and quantitative investigations *in vitro* of the amino-acid make-up of the proteins concerned.

#### Relation between Chemical Constitution of the Proteins and Their Food Value

The amino acids which result from the digestive or other hydrolysis of food proteins differ so widely in their chemical constitution (Chapter IV) as to make it improbable that they could all function interchangeably in all the chemical reactions which the nutritional process involves. Yet it is easily conceivable that some of the simpler amino acids might be formed in the body either synthetically or in the course of metabolism of some of those of larger molecular weight, in which case they need not be supplied by the food.

Obviously all amino acids which are essential to the structure

of body proteins are in this sense nutritionally essential substances; but the term *nutritionally essential* is often used to indi-

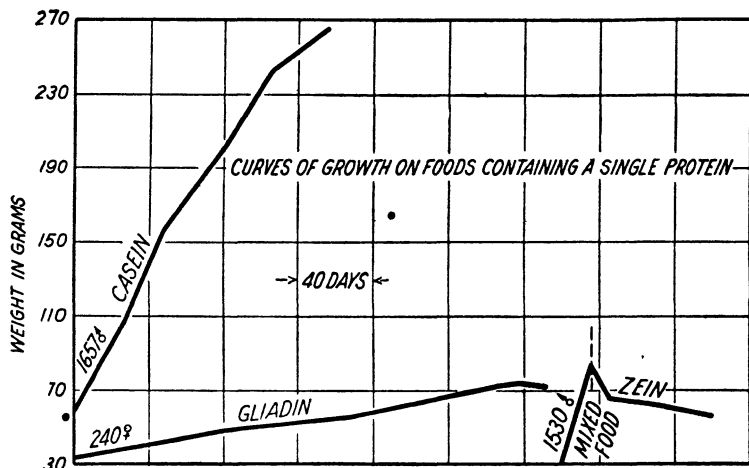


FIG. 1. — Showing typical curves of growth of rats on diets otherwise similar and adequate but containing in each case only a single protein: casein, gliadin, or zein. (Courtesy of Dr. L. B. Mendel and the *Journal of the American Medical Association*.)

cate that the substance in question *must be fed* (furnished by nutriment consumed).

The nature of the experimental evidence as to whether or not certain individual amino acids are nutritionally essential may now be considered briefly.

*Lysine* and *tryptophane* may conveniently be discussed together, because of the evidence furnished for these two amino acids simultaneously by the work of Osborne and Mendel. In experiments in which young rats received food mixtures containing 18 per cent of casein, gliadin, or zein, respectively, as the sole protein fed, it was found that casein sufficed not only for maintenance but also for excellent growth; gliadin sufficed for maintenance but for only very slight growth; zein did not suffice even for maintenance (Fig. 1). Gliadin is now known to contain a small amount of lysine; and an additional small (and presumably constant) amount was introduced into both the gliadin and zein rations with the material used to supply the water-soluble vitamins. These facts were not appreciated in the earlier interpretation of the results; but, when properly understood, do not materially detract

from the value of the experiments, at least for the purpose of this discussion.

Supplementary experiments showed that more lysine was needed to permit normal growth on the gliadin diet; while the zein diet required the addition of tryptophane to make it adequate for maintenance and the addition of more lysine to make it adequate for growth (Fig. 2). This evidence, combined with that

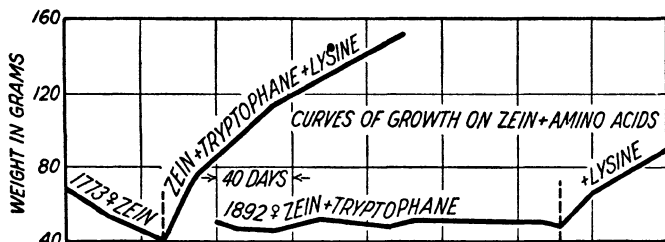


FIG. 2. — Showing the effect of adding tryptophane or tryptophane and lysine to a diet containing zein as the sole protein. (Courtesy of Dr. L. B. Mendel and the *Journal of the American Medical Association*.)

of many other experiments all indicating that no other amino acid could take the place either of lysine or of tryptophane, finally made it evident that lysine and tryptophane must each be furnished by the food if nutrition is to be adequately supported.

*Cystine* and *methionine* are also best studied together. Osborne and Mendel showed that although casein fed as 18 per cent of the diet supported a normal rate of growth, when fed at a 9 per cent level it permitted only about one-half as rapid growth. In this case the limiting factor was not lysine but cystine, for the addition of cystine to the low-casein diet induced a normal rate of growth, which was immediately checked when the cystine was withdrawn and resumed when the cystine was again added to the ration (Fig. 3). Later, another sulfur-containing amino acid, methionine, was discovered in proteins, and found to be nutritionally essential. In further experiments by Rose and coworkers it has appeared that methionine can function as precursor to supply the needed cystine; but that cystine does not supply the needed methionine, or only to a limited extent if at all.

Thus cystine is apparently dispensable and methionine indispensable in this sense; and as the term nutritionally essential has been commonly used in grouping the amino acids, methionine

would be counted as nutritionally essential and cystine would not. Yet the cystine intake may actually be the determining factor in growth as in some of Osborne and Mendel's experiments. This is

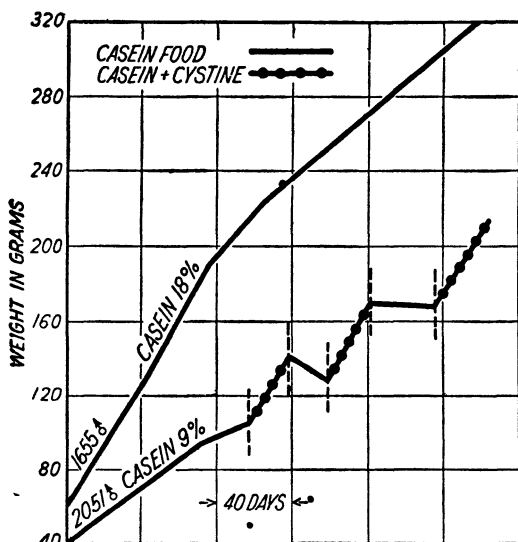


FIG. 3. — Showing an experiment in which the insufficiency of a low-casein diet was corrected by increasing the intake of cystine. (Courtesy of Dr. L. B. Mendel and the *Journal of the American Medical Association*.)

too important a fact to be minimized or lost sight of. Hence to merely memorize lists of amino acids as being or not being “dispensable,” or “nutritionally essential” in the special sense so often used, may easily be somewhat misleading.

### Which Amino Acids Are Indispensable Nutrients?

To keep in mind the special meanings given to certain terms in this connection, let us recall that W. C. Rose and his coworkers have demonstrated that a suitable mixture of amino acids is a nutritionally satisfactory substitute for protein even in the diet of rapidly growing experimental animals. He has shown also that if any one of ten so-called “nutritionally essential” or “indispensable” amino acids is lacking in the amino acid mixture, normal growth will not occur. But if all ten of these are provided in suitable amounts, the body can form from them the remaining amino acids which enter into the composition of its proteins. The

ten amino acids now called "indispensable," or "nutritionally essential" in the sense that they must be furnished through the nutriment, are:

|            |               |
|------------|---------------|
| Arginine   | Methionine    |
| Histidine  | Phenylalanine |
| Isoleucine | Threonine     |
| Leucine    | Tryptophane   |
| Lysine     | Valine        |

A few words in regard to each of these "indispensable" amino acids may help toward the scientific discrimination which is so much more desirable than merely to memorize, as a group, ten substances each of which has its own interesting character and significance.

*Arginine* seemed almost dispensable, yet on diets very carefully freed from it young animals did not grow at a fully normal rate.

*Histidine* at first appeared possibly to be interchangeable in the diet (because interconvertible in the body) with arginine; but further experiments indicated that histidine is more probably an indispensable nutrient in the special sense in which the term "indispensable" or "nutritionally essential" is used in this connection. (In another sense, histidine is not strictly indispensable for if its imidazole nucleus is supplied as the corresponding alpha-hydroxy or alpha-keto acid, under suitable conditions the alpha-amino group may be substituted and the histidine thus formed in the body.)

*Isoleucine*, *leucine*, *phenylalanine*, and *valine* are apparently the most typical cases of amino acids long known as constituents of body tissues, but whose status as indispensable nutrients was established only with the development of Rose's techniques of experimental feeding.

*Lysine* and *tryptophane* had, as noted above, been established as individually indispensable in earlier researches; and in these cases the empirical findings were clearly interpretable in terms of the unique structures of their respective molecules.

*Methionine*, with its relation to cystine which has been discussed briefly above, constitutes a case of a somewhat different kind.

*Threonine* is unique in the fact that it remained undiscovered until Rose in his studies of these very problems of dispensability

*versus* indispensability of individual amino acids found evidence of the presence (in certain proteins and their digestion-products) of a nutritionally essential factor not previously known.

A clearer and more discriminating view may also be obtained if we here add two well-investigated illustrations of amino acids which while having indispensable functions in the body's nutritional processes are yet *not* "*nutritionally essential*" in the special sense which we have just been studying:

*Glycine*, although an essential constituent of body tissue, need not be furnished by the food, for proteins which do not yield glycine on hydrolysis have been shown to be adequate when fed as sole protein of an experimental ration. It appears therefore that supplies of glycine fully adequate to meet all *normal* needs may be formed within the body itself; but the ability to form it is not unlimited, as Griffith has shown. When young rats are fed relatively large amounts of sodium benzoate, the benzoyl radicle of which they conjugate with glycine and excrete as hippuric acid, growth may cease because this extra demand for glycine exceeds the ability of the body to form the amino acid. Under these conditions, the addition of extra glycine to the diet results in a resumption of growth, indicating that the cessation has been, in fact, due to a glycine deficiency.

*Alanine* is probably also formed in the body in ample amounts for normal needs. Feeding experiments with food supplies adequate in other respects and lacking alanine may not seem necessary because alanine is a constituent of all the food proteins which have thus far been studied in detail with reference to their amino acid make-up. There is, moreover, experimental evidence (Chapters VII and XI) that the body can make alanine from lactic or from pyruvic acid, and as one or both of these latter may be regarded as constantly being formed in the intermediary metabolism of carbohydrate, this would probably provide the body with a source of alanine whether it were furnished as such by the food proteins or not.

As briefly noted above, some of the amino acids which are nutritionally essential in the sense that the body cannot synthesize them *de novo* or from substances normally occurring in the food, may be formed in metabolism from specific closely-related substances if these are supplied. For example, growth in animals stunted by histidine deficiency is resumed when imidazole lactic



or imidazole pyruvic acid is fed; and similarly, indole lactic or indole pyruvic acid may successfully replace tryptophane in the diet of rats; but the substituted lactic and pyruvic acids corresponding to the amino acid lysine have apparently no favorable effect in relieving lysine deficiency. Thus, presumably, the body (of the rat) can replace the  $\alpha$ -hydroxyl or  $\alpha$ -carbonyl group by an amino group to form histidine or tryptophane, but not to form lysine. Or, as regards histidine and tryptophane, the indispensable part is the unique heterocycle of each, and not the alanine sidechain, lactic and pyruvic acids being interconvertible with alanine (Chapter VII).

While much remains to be done in this field, the general plan of experimentation and interpretation initiated and carried to such an important series of correlations between chemical structure and nutritional function by Osborne and Mendel, and more recently by Rose and coworkers, already constitutes one of the most important advances in the development of the chemistry of food and nutrition.

Other branches of the same general line of research are the feeding of individual proteins or the protein mixtures of individual foods as the sole protein of the diet and the study of the supplementary relationships in nutrition between individual proteins or the natural protein mixtures of different foods.

### **Classification of Proteins as to Nutritional "Completeness"**

To emphasize the differences between individual proteins they have sometimes been grouped as:

A. "Complete": Maintaining life and providing for normal growth of the young when used as a sole protein food. Examples of these are: Casein and lactalbumin from milk; ovalbumin and ovovitellin of egg; glycinin of soy bean; excelsin of Brazil nut; edestin, glutenin, and maize glutelin of the cereal grains.

B. "Partially Incomplete": Maintaining life but not supporting normal growth. Gliadin of wheat is a well-demonstrated example of this class. Hordein of barley and the prolamin of rye are similar in nutritive value to gliadin.

C. "Incomplete": Incapable either of maintaining life or of supporting growth, when fed as the sole protein. Zein of corn (maize) and gelatin are the conspicuous examples.

Any such grouping of the proteins, however, should be used

with much discrimination, and with great care to insure an understanding of the quantitative aspects of the experimental data, if misconceptions are to be avoided. Edestin is a conspicuous example of a "complete" protein, having served as the sole protein food of a family of rats for three generations; but when the percentage of edestin in the food mixture was considerably reduced, results like those above described for gliadin were obtained — the diet did not support a normal rate of growth, but this could be secured by adding lysine to the food mixture. Similarly casein when fed in reduced proportion to the total food mixture did not support normal growth; but growth became normal when cystine was added. Thus "complete" proteins may behave as "partially incomplete" when fed in reduced proportion. It is also to be remembered that varying rates of growth in different species (not to mention other differences) make inadmissible any broad generalizations as to the proportion in which any protein should be fed to species other than that with which its "completeness" or "incompleteness" has been demonstrated.

In 1916, Osborne and Mendel published quantitative measurements of the relative efficiency (for support of growth in young rats) of some of the "complete" proteins. The rate of gain obtained with 8 per cent of lactalbumin required 12 per cent of casein or 15 per cent of edestin; or, as they also state the results, "to produce the same gain in body weight 50 per cent more casein than lactalbumin was required, and of edestin nearly 90 per cent more."

### **Supplementary Relations between Proteins in Nutrition**

In the feeding experiments shown in Fig. 1, each ration contained only a single isolated protein. This is the ideal condition for the experimental comparison of individual proteins, but is quite different from the ordinary conditions of practice, since our common protein foods all contain mixtures of proteins, so that even if only a single article of food were consumed the diet would still furnish more than one protein at a time. By feeding definite mixtures of pure proteins Osborne and Mendel have beautifully demonstrated the way in which proteins supplement each other in nutrition. Thus zein is, as we have seen, inadequate as a sole protein food; lactalbumin is adequate when fed in sufficient quantity but when constituting only 4.5 per cent of the food mix-

ture of rats it supports only slow growth; but a food mixture containing 4.5 per cent of lactalbumin and 13.5 per cent of zein supports growth at a fully normal rate (Fig. 4). This shows that a relatively small amount of lactalbumin (one fourth of the protein fed) sufficed to furnish the amino acid groups which the zein lacked. It shows also that zein, which when fed as a sole protein

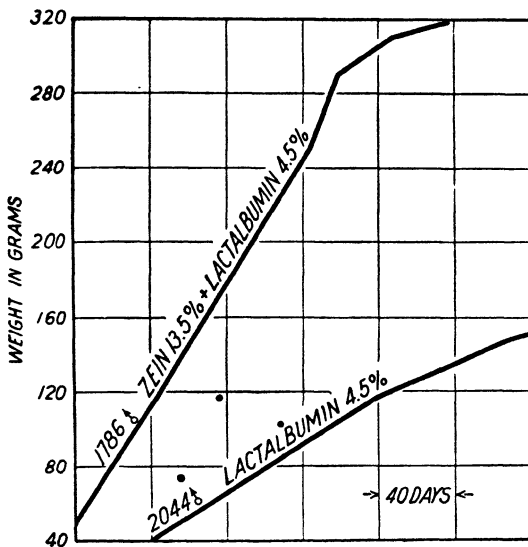


FIG. 4. — Showing the efficiency of lactalbumin as a supplement to zein, and also that zein may take an important part in growth although zein *alone* is inadequate either for growth or maintenance. (Courtesy of Dr. L. B. Mendel and the *Journal of the American Medical Association*.)

is insufficient even for maintenance, is able as a constituent of a proper food mixture to take part in supplying the materials for growth, to such an extent as to more than double the growth-rate. Thus zein, although inadequate for either maintenance or growth when isolated and fed alone, may nevertheless take an important part in both maintenance and growth when fed as a part of a proper mixed diet. Moreover it may not even be necessary to resort to a mixture of food materials in order to make good the deficiencies of the individual incomplete protein. Corn (maize) itself contains, along with zein, an almost equal amount of another protein, maize glutelin, which Osborne and Mendel have shown to be capable of supporting a normal rate of growth.

Similarly the other proteins of the wheat kernel supply in more liberal proportion the lysine in which gliadin is poor, so that (as Osborne and Mendel and also McCollum, Simmonds, and Parsons have shown) with ground whole wheat as the sole source of protein good growth can be obtained.

Thus it is plain that the mixtures of proteins contained in different articles of food as we eat them do not differ in such a striking way as do the individual proteins when isolated and fed singly; but neither is it true that the proteins of different articles of food are equivalent for all practical purposes. Hart, McCollum, and their associates have shown that the natural protein mixture of milk is much more efficient than an equal weight of the mixed proteins of grain, both for the support of growth and as food for the production of milk in dairy cattle. While it is always possible that in comparisons between natural food materials the results *may* be influenced by differences in the unknown food constituents which may be present, yet in the cases here cited there is ample ground for confidence that the differing efficiencies ascribed to milk and grain proteins are mainly due to the same differences of chemical constitution (amino-acid make-up) to which are attributable the striking results obtained in the experiments previously cited in which isolated proteins were fed.

This is made all the more certain by the fact that subsequent experiments by Steenbock and Hart have demonstrated that the protein mixture of a properly proportioned ration of grain and milk may show high nutritive efficiency, doubtless because milk proteins are rich in lysine and tryptophane, which are the amino acids most effective in supplementing the proteins of the cereal grains.

### **Amino-Acid Derivatives as Hormones or Nutritional Catalysts**

Both by Willcock and Hopkins and by Osborne and Mendel it was suggested that nutritionally essential amino acids may function not merely as "building stones" to be used along with other amino acids in the growth and replacement of tissue protein, but also in more individual and specific ways as precursors of hormones or other specifically essential substances in the body.

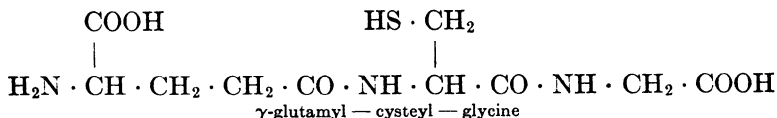
Even the growth experiments of the types already cited lend support to this general view, for of the amino acids now regarded as nutritionally essential, some seem to be more urgently and

"vitally" essential than others: a shortage of cystine or lysine results in suspension of growth with surprisingly little if any injury; whereas an animal subjected to a correspondingly drastic shortage of histidine or tryptophane not only stops growing but soon shows signs of torpidity, lack of tone, or actual illness.

### Glutathione

Investigation by Hopkins and others has revealed the presence in active plant and animal tissues of an amino-acid derivative which he names glutathione. This is a tripeptide of glutamic acid, cysteine, and glycine. It appears to be an important factor in the oxidation and reduction reactions of the cell, probably by virtue of the reversible change between the sulfhydryl groups ( $-\text{SH}$ ) of the *cysteine* radicles in the glutathione and the disulfide groups ( $-\text{S}-\text{S}-$ ) of the *cystine* radicles in the oxidized product. When glutathione is oxidized, two molecules containing cysteine unite to form one molecule of the oxidized product containing cystine. There is evidence that this reaction is catalyzed by traces of heavy metals such as iron and copper possibly in conjunction with some, as yet unidentified, non-metallic substance.

The mode of linkage of the three amino acid radicles in glutathione and its corresponding structural formula, based upon its reactions and properties, were reported independently by Nicolet and by Kendall, Mason, and McKenzie, in 1930, to be



Glutathione is readily soluble in water and gives an intense nitroprusside reaction which is characteristic of all active plant and animal tissues. This test consists, in general, of treating a suspension of tissue or an extract with potassium nitroprusside in the presence of ammonia. When the test is positive, a color resembling that of potassium permanganate is developed. It is probable that a reduction of the nitroprusside molecule takes place. Cysteine which contains the sulfhydryl group ( $-\text{SH}$ ) gives this test whereas its oxidation product, cystine ( $-\text{S}-\text{S}-$ ), does not. Because of this, the reducing property of tissues was, even before the isolation of glutathione, more or less commonly ascribed to the sulfhydryl group, but until the work of Hopkins

no experimental proof of this was available as cysteine was known to be too reactive to exist to an appreciable extent in the tissues. This property now appears to be largely due to the sulfhydryl groups ( $-\text{SH}$ ) of the glutathione molecule. As the oxidized ( $-\text{S}-\text{S}-$ ) form does not give the nitroprusside reaction, the oxidation and reduction of glutathione may readily be followed by means of this test.

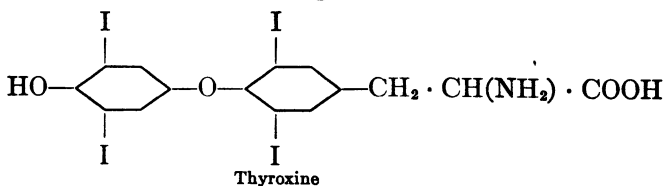
Hopkins has pointed out that, "Equilibrium in the living cell would seem to be such that the greater part of the substance (glutathione) present exists in the reduced condition; but oxidation and reduction of the constituent sulphur groups are reversible processes in the tissues, and both forms may at any moment be present." ". . . factors are present in the tissues which promptly reduce the oxidized product whenever its concentration is raised above an equilibrium value."

Thus, while cysteine is too unstable and reactive to exist, to an appreciable extent, as such in the tissues, it is protected in glutathione, and furnishes the reactive sulfhydryl group of this important compound. Glutathione appears to be resistant to the proteolytic enzymes of the body and to exist to an appreciable extent in all active tissues. In addition to protecting the cysteine from metabolic processes, glutathione furnishes an oxidation-reduction system which is readily soluble.

### Thyroxine

Thyroxine, an iodine-containing amino-acid derivative, formed in the thyroid gland and carried thence to the tissues of the body as a whole where it increases the rate of oxidation (energy metabolism), was isolated by Kendall in 1914 (Fig. 5).

In 1926, Harington published a method for greatly increasing the yields of thyroxine obtainable from the thyroid gland and a much more comprehensive study of its properties than had previously been possible. This led to the synthesis of thyroxine which was accomplished by Harington and Barger in 1927. Its structure as thus established is given below:



Thyroxine is, therefore, probably a derivative of tyrosine. The original papers listed at the end of the chapter and the monograph by Kendall (1929) may be consulted for more detailed accounts of this important substance.

The very important relations of thyroxine to the rate of oxidation in the body, and to development and health, are discussed

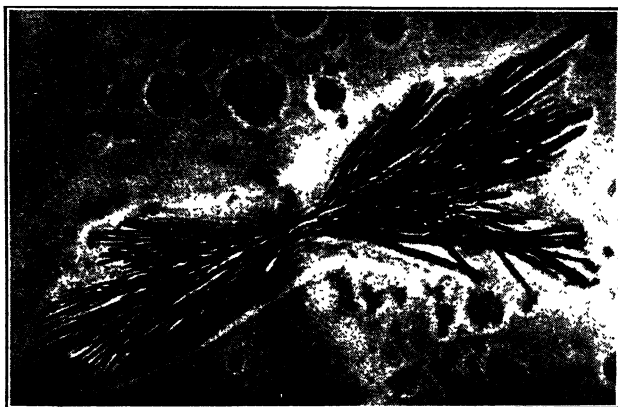


FIG. 5. — Crystalline thyroxine. (Courtesy of Dr. E. C. Kendall.)

in Chapters IX and XVI in connection with the study of the conditions affecting the energy metabolism and the functioning of the thyroid gland from the standpoint of the iodine metabolism, respectively.

### Adrenine, Adrenaline, or Epinephrine

The substance called adrenine, adrenaline, or epinephrine is the active substance formed by the adrenal (suprarenal) glands. There are differences of opinion as to its part in the oxidation processes of the body, or in the mobilization of oxidizable material. Its influence upon the rate of oxidation in the body is less marked than that of thyroxine and also more rapid and transitory. Following an injection of adrenaline there is an almost instantaneous increase in the basal metabolic rate which rapidly reaches a maximum and then soon drops back to normal. The entire effect, on the basal metabolic rate, of administration of epinephrine in the normal adult, is over in a few hours; whereas with thyroxine there is a well-defined delay, six to eight hours, before the basal

metabolic rate is increased, and the effects of a single injection upon the basal metabolic rate may still be observable for five or six weeks after the injection. Boothby has found that the extent of the increase in the rate of oxidation following the administration of epinephrine is dependent upon its concentration in the tissues. The other functions of epinephrine in the body will not be discussed here.

Epinephrine has been isolated and synthesized. It is dihydroxyphenyl hydroxyethyl methyl amine. Hence it is related in structure to, and is probably a derivative of, tyrosine.

### Insulin

Insulin is sometimes spoken of as being concerned in the burning of glucose in the body. This appears to be true in the sense that insulin helps to prepare glucose for the intermediary metab-



FIG. 6. — Hexagonal crystals of insulin. (Courtesy of Dr. J. J. Abel.)

olism which may lead to oxidation; not in any sense which would imply that insulin is an oxidizing agent.

The most direct chemical evidence on the subject of the action of insulin is that it causes a lowering of the concentration of



glucose in the blood; and this is observed both in the diabetic and in the normal body. This furnishes an experimental method by which the characteristic potency of insulin concentrates can be measured and progress toward the isolation of the pure substance may be judged.

With this criterion as a guide, investigators in several laboratories have been working actively upon the chemistry of insulin. Abel obtained it in crystalline form (Figs. 6 and 7) in 1926 and



FIG. 7. — Prismatic crystals of insulin. These crystals were all beautifully transparent, the appearance here suggesting opaqueness being due simply to difficulties of focus. (Courtesy of Dr. J. J. Abel.)

later extended this phase of the work. Many other investigators in different laboratories, using the Abel method, have confirmed the findings. Harington and Scott (1929), using a somewhat different method, found that their crystals were identical in appearance with the crystals isolated by Abel. The results of a study of four samples of crystals, two of which were prepared by the method of Abel and two by the method of Harington and Scott, showed a uniform potency and the chemical analyses agreed well.

Crystalline insulin gives the typical biuret, Millon, Pauly, ninhydrin, and xanthoproteic reactions, as well as specific color reactions for arginine, cystine, and tyrosine. Thus insulin, like typical proteins, is an amino-acid derivative. Furthermore, physico-chemical, chemical, and enzymatic studies of the most diverse sorts all confirm and strengthen the classification of this crystalline substance as a typical protein. The molecular weight as indicated by the ultracentrifuge technique is about 35,000. Jensen and Evans (1934) summarize very convincing evidence that the hypoglycemic factor is identical with the crystalline protein, and not merely adsorbed or loosely bound to it. These authors have also (1935) pointed out that "practically the whole of this protein molecule" may be accounted for in terms of a relatively few amino acids: tyrosine (12%); cystine (12%); glutamic acid (21%); leucine (30%); arginine (3%); histidine (8%); lysine (2%); phenylalanine and proline; and that the most careful studies have failed to reveal any special non-amino-acid constituent (prosthetic group) which would explain the unique physiological action of crystalline insulin.

The hypoglycemic effect of the hormone must therefore depend, if not on the entire protein molecule, at least on some certain groupings of amino acids in the molecule.

Several publications upon insulin are included among the suggested readings listed below.

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## CHAPTER VI

### ENZYMES AND DIGESTION

THE CARBOHYDRATES, fats, and proteins as they exist in foods are in most cases not of a nature to be used by the body tissues in the exact form in which they are eaten, but must usually undergo more or less alteration in the digestive tract to fit them for absorption and assimilation. In so far as the changes which the food undergoes in the alimentary tract are chemical they are brought about mainly by the action of digestive enzymes.

#### Classification and General Properties of Enzymes

The word *enzyme* (from the Greek "in yeast") was introduced by Kühne as a general designation for the substances formed in plants or animals which had previously been called *soluble* or *unorganized* ferments to distinguish them from *organized* ferments (fermentation organisms). As more and more of the activities previously regarded as characteristic of organisms have been found to be due to enzymes, the conception of enzyme action has broadened until now enzymes are commonly defined as "catalysts formed in plant or animal cells." Although each enzyme is generally supposed to be a definite chemical substance, the identification and classification of enzymes is based upon the changes which they bring about. Some of the better-known groups of enzymes are as follows:

1. The hydrolytic enzymes.
  - a. Proteolytic or protein-splitting enzymes (proteases).
  - b. Lipolytic or fat-splitting enzymes (lipases).
  - c. Amylolytic or starch-splitting enzymes (amylases).
  - d. Sugar-splitting enzymes (sucrase, maltase, lactase).
2. The coagulating enzymes, such as thrombin or thrombase, which assists in the clotting of blood, and rennin, which causes the clotting of milk.
3. The oxidizing enzymes (oxidases, dehydrogenases).
4. The reducing enzymes (reductases).

5. Those which, like the zymase of yeast, produce carbon dioxide without using free oxygen.

6. Enzymes causing the breakdown of a larger into a smaller molecule of the same composition, as in the production of lactic acid from glucose.

7. Enzymes causing chemical rearrangement without breaking down of larger into smaller molecules (mutases).

*Terminology of the hydrolytic enzymes.* Except in so far as some familiar enzymes continue to be known by their old established names (pepsin, rennin, trypsin, etc.), scientific usage now generally follows the suggestion of Duclaux that each hydrolytic enzyme be designated by a name indicating the kind of substance on which it acts, together with the suffix *ase*. Thus starch-splitting enzymes are called *amylases*; fat-splitting enzymes, *lipases*; protein-splitting enzymes, *proteases*. The name showing the kind of change brought about or catalyzed by the enzyme is often preceded by an adjective to indicate its source; e.g., *salivary amylase* (ptyalin), *pancreatic amylase* (amylopsin). Such designation does not necessarily imply that the amylase found in the saliva either is or is not the same substance as the amylase of the pancreatic juice. Whether enzymes from various sources which have the same specific catalytic activity are identical chemically has been investigated in the case of several highly purified enzymes. From recent work it appears, for instance, that the crystalline pepsins from cattle and from swine are different substances; also the crystalline trypsins from these two species. It has also been shown that pancreatic amylase differs from malt amylases, and that these latter differ also from one another.

In discussions of enzyme action the substance on which the enzyme acts is commonly called the *substrate*.

Within the cell producing it, an enzyme often exists in an inactive form known as the *zymogen*, or antecedent of the active enzyme. The zymogen may be stored in the cell in the form of material which is converted into active enzyme at the time of secretion, or the secretion may be poured out with the zymogen not yet completely changed to active enzyme, or sometimes in a form which requires the presence of some other substance in order to render it active. In this case the latter substance is said to *activate* the enzyme. Activation may also mean a simple increase of activity.

*Prominent among chemical studies of enzymes* are: (1) attempts to ascertain what factors influence, or are necessary for, their activity; and (2) attempts to concentrate, purify, or isolate the active material. The second of these necessarily depends largely upon the first because enzymes are known only by their characteristic (catalytic) activities and any thoroughly satisfactory attempt to study them must involve quantitative measurements of their action. Solutions containing enzymes may readily be obtained from plant or animal tissues by extraction with suitable solvents. Thus, for example, the enzymes of the pancreas may be obtained in solution by grinding and extracting the gland. Such extracts contain many substances which were naturally present with the enzyme, some of which may be necessary for its stability in solution or for its normal activity. These necessary substances must be supplied in proper concentrations when the purified material is examined for activity. Among the many factors which are known to influence enzyme action markedly is the hydrogen ion activity of the enzyme-substrate system. For most enzymes this must be adjusted within rather narrow limits if optimal results are to be obtained; and in some cases even to obtain any positive result. This hydrogen ion activity is commonly referred to in terms of pH, which is the symbol introduced by Sørensen to represent the negative logarithm of the hydrogen ion concentration (activity), or  $\text{pH} = \log \frac{1}{[\text{H}^+]}$ .

As the hydrogen ion activity most favorable to the action of an enzyme depends upon many interrelated factors, it should not be stated dogmatically. Remembering this, it is nevertheless of interest to note the values which have been reported as being most favorable for the action of a few of the typical enzymes. Thus, invertase (sucrase) from yeast has been reported to be most active in solutions of about pH 4.4 (Nelson), while that from the intestinal digestive juice has been found to be most active in solutions of about pH 7.0. The amylases of barley malt are most active in solutions of about pH 4.5, while that from the pancreas exerts its maximum activity in solutions of about pH 7.0 to 7.2.

With some enzymes the optimal pH is different for different substrates. Thus pepsin acting on gelatin shows a pH optimum of about 2.4, whereas when acting on casein the optimum is about pH 1.8 (Northrop, 1922). Here, and also in the case of trypsin,



the changes in activity with pH seem to be related to the state of ionization of the substrate, the acid salt of the protein being most readily attacked by pepsin, the alkali salt by trypsin (Northrop, 1922). An interesting interpretation of the effect of hydrogen ion activity on enzyme activity was suggested by Michaelis for invertase (sucrase). He pointed out that the curve relating reaction velocity to pH is very similar to that relating the concentration of the unionized fraction of an amphoteric electrolyte to pH, with the optimum hydrogen ion activity for the enzyme corresponding to the isoelectric point of the ampholyte. He suggested that invertase is in fact an ampholyte of which only the unionized form is catalytically active. A similar relation was found with several other enzymes, while in some cases the active form of the enzyme appeared to be ionic. Using a direct and ingenious method, Northrop (1924, 1925) showed that trypsin behaves as a univalent positive ion between pH 2 and 10, and pepsin as a univalent negative ion between pH 1 and 7. With these enzymes, the changes in activity with pH seem to be related to the state of ionization of the substrate.

### Activities of the Digestive Enzymes

That the typical digestive enzymes are very pronounced catalysts may be judged from the relatively large amounts of material which they are capable of digesting under favorable conditions. Thus, pancreatic amylase as highly purified by modern methods tends (as stated above) to lose its activity rather rapidly when in solution, yet in 30 minutes at 40°, one part of purified pancreatic amylase hydrolyzed about 20,000 parts of starch and formed about 10,000 parts of maltose (along with about an equal, unmeasured, amount of dextrin); and in longer experiments it hydrolyzed 4,000,000 times its weight of starch with the formation of 2,800,000 times its weight of maltose before it had all become inactivated. This marked enzymic activity was exhibited by the preparation at a dilution of 1:100,000,000 parts of water. The most delicate tests for proteins are not valid at dilutions greater than about 1:100,000. The preparation reacted like typical protein to the usual protein tests, but its own enzymic activity constituted a test for its presence which was 1000 times more delicate. Thus the failure of protein reactions in solutions enzymically active does not show that no protein is present or that the enzyme is of

other than protein nature in its chemical composition, although this negative conclusion was erroneously drawn by some investigators and repeated by many writers.

It may perhaps be asked why, if enzymes act by catalysis, there should be any limit to the amount of substrate which the enzyme can hydrolyze. One reason that enzymes cannot hydrolyze infinite amounts of substrate is that they are themselves unstable organic substances which undergo decomposition when kept in solution. In most cases, the purer the enzyme the more rapidly its solutions lose their activity. Another reason that an enzyme does not continue to hydrolyze substrate indefinitely is that the reaction is progressively retarded by the accumulation of the products formed, — though this latter factor seems much more influential in some cases than in others, and in some instances is hardly demonstrable.

The activity of certain enzymes may be stopped, even when all other conditions are favorable, by the accumulation of the products of the reaction; and, in certain circumstances, the action of the enzyme may be reversed so as to accelerate a change in the opposite direction to that in which it ordinarily acts. Thus Croft Hill showed it to be possible to reverse the ordinary action of maltase so as to make it bring about a conversion of mono- into di-saccharide; Pottevin synthesized triolein by means of pancreatic lipase; Taylor and others have demonstrated a partial reversion of the tryptic digestion of proteins; and Wasteneys and Borsook have carried out a very extended and systematic investigation of the reversion of peptic and tryptic digestion. Enzymes are widely distributed in the body and many of the transformations which take place in the course of metabolism are best explained on the ground of the reversibility of enzyme action.

At this point it may be convenient to summarize in tabular form the occurrence and action of the chief digestive enzymes. (See summary on next page, which, as the reader will note, relates only to the enzymes which function in the digestion of the food. Undoubtedly there are analogous enzymes in the bodily tissues which catalyze some of the processes described in Chapters VII and XI. Also there are the tissue respiratory enzymes mentioned in later chapters.)

## SUMMARY OF CHIEF DIGESTIVE ENZYMES

| ENZYMES              |   | SECRETED BY                 | ACTION  |
|----------------------|---|-----------------------------|---|
| Act on Carbohydrates | Ptyalin (salivary amylase)              | Salivary glands             | Converts starch to maltose  |
|                      | Amylopsin (pancreatic amylase)          | Pancreas                    | Converts starch to maltose  |
|                      | Invertase (Sucrase)                     | Intestinal mucosa           | Converts sucrose to glucose and fructose  |
|                      | Maltase                                 | Intestinal mucosa           | Converts maltose to glucose   |
|                      | Lactase                                 | Intestinal mucosa           | Converts lactose to glucose and galactose   |
| Act on Fats          | Lipases                                 | Gastric mucosa and pancreas | Split fats to fatty acids and glycerol *  |
| Act on Proteins      | Pepsin                                  | Gastric mucosa              | Splits proteins to proteoses and peptones <sup>a</sup>                            |
|                      | "Trypsin" (actually a group of enzymes) | Pancreas                    | Splits proteins to proteoses, peptones, polypeptides and amino acids <sup>b</sup> |
|                      | "Erepsin" (actually a group of enzymes) | Intestinal mucosa           | Splits peptones to amino acids and ammonia <sup>c</sup>                           |

\* When pepsin is allowed to act for many hours, lower protein breakdown products such as poly- and dipeptides and even free amino acids may be liberated, but under normal conditions of digestion *in vivo* the hydrolysis of protein by pepsin is not so extensive.

<sup>b</sup> Northrop has isolated from pancreas two crystalline proteolytic enzymes which he has called *trypsin* and *chymotrypsin*, both of which differ from earlier preparations of "trypsin."

<sup>c</sup> The catalytic reactions attributed to "erepsin" have more recently been attributed to a number of different enzymes occurring together.

## The Chemical Nature of Some Typical Enzymes

In 1902 Pekelharing prepared a specimen of purified pepsin, which product contained carbon, hydrogen, nitrogen, and sulfur in proportions within the range of variation found among ordinary proteins. It also behaved like ordinary proteins in the xanthoproteic test and Millon reaction and in showing the presence of the tryptophane group.

Dezani, in 1910, carried forward the work upon the chemical

nature of pepsin by preparing what was believed to be a substantial duplicate of Pekelharing's product and submitting this to hydrolysis, followed by search for individual hydrolytic products according to the methods which had recently been developed in the study of the structure of the proteins. He demonstrated the presence of leucine, tyrosine, arginine, histidine, and lysine and also found evidence of other amino acids which the limitations of his material and methods did not permit him to identify.

Northrop (1929, 1930) has further confirmed the protein nature of pepsin by physico-chemical study of crystalline pepsin. Thus pepsin as prepared by several investigators is a nitrogenous material not identical with any other known substance but complying with the criteria of our present conception of a protein in elementary composition, in color reactions, and especially in yielding the familiar amino acids upon hydrolysis.

Even earlier than Pekelharing's work on pepsin, Osborne (1895) had published an investigation of the chemical nature of diastase (malt amylase), which may be regarded as marking the beginning of our modern knowledge in this field, though recognition followed so tardily that its importance is perhaps not adequately reflected in the literature of the subject. From this work it appeared that the enzymic activity is a property of a definite fraction of the protein material of the malt, or in other words that the enzyme is protein in its chemical nature.

Following the confirmation of Osborne's work on malt amylase, pancreatic amylase also has been studied by modern methods with reference to its chemical nature.

In an investigation \* in which attempts at purification were guided and their success largely judged by quantitative determinations of the enzymic activity of the products, there was developed a method of purification which in numerous independent experiments yielded material that was not only extraordinarily active in the hydrolysis of starch but was essentially uniform both in enzymic activity and in chemical nature. Such a result in itself indicates strongly that this material represents at least some approximation to an actual isolation of the enzyme, and this finding has been confirmed by several independent methods of investigation, including finally the crystallization of the purified

\* *Journal of the American Chemical Society*, **33**, page 1195; **34**, page 1104; **35**, page 1790; **48**, page 2947. *Journal of Biological Chemistry*, **88**, page 295.

substance. These preparations show the composition and color reactions of typical proteins and, like Osborne's malt amylase, the material when heated in water solution yields an albumin coagulum and a proteose or peptone which remains in solution. Moreover, on hydrolysis the material yields the same groups of amino acids which are yielded by typical proteins such as casein, which it also resembles in elementary composition.

Although the amylase of the saliva has not been so extensively studied, it appears, from recent work of Tauber and Kleiner (1934) to be also of protein nature, since ptyalin preparations lost their enzymic activity when acted upon by proteases.

Highly purified preparations of pancreatic lipase give typical protein color tests, and in solubility and precipitation reactions this enzyme has the characteristics of a globulin (Glick and King, 1933).

Other enzymes and zymogens have been crystallized (Figs. 8 to 10) and found to behave as typical proteins in so far as they

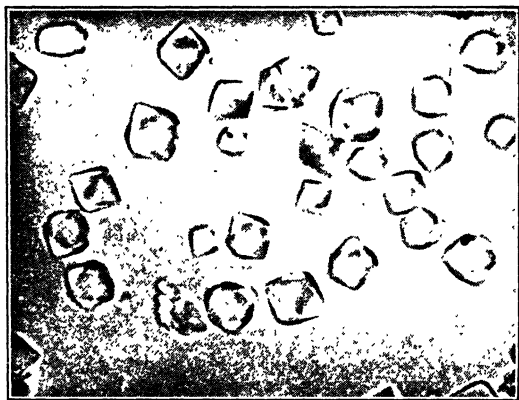


FIG. 8. — Crystalline urease. (Courtesy of Dr. J. B. Sumner.)

have been studied; and the very thorough investigations of crystalline preparations of urease, pepsin, and trypsin have served to demonstrate that the catalytic activity resides in and depends upon the integrity of the protein molecule. The slightest change in the native protein molecule which is detectable by the most delicate physico-chemical measurements is attended by a loss in activity directly proportional to the fraction of native protein that has been altered in this way.

In the case of the typical enzyme malt amylase, the enzymic activity has been found to be so inseparable from the particular protein in which it resides as to migrate with it under the influence of the electric current in either direction according to the hydrogen ion activity of the solution. Thus this enzyme shows, like other protein substances, a characteristic isoelectric point. For further discussion of the significance of this observation, both as added evidence of the protein nature of the enzyme and as throwing light

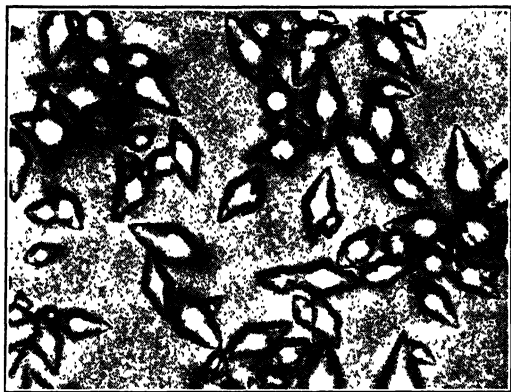


FIG. 9 — Crystalline pepsin. (Courtesy of Dr. J. H. Northrop.)

upon the conditions which influence the enzymic activity, the reader is referred to the original paper (Sherman, Thomas, and Caldwell, 1924).

There is also much indirect evidence of several independent kinds supporting the view of the protein nature of some typical enzymes. In addition to the large amount of evidence derived from investigations upon the purification of typical enzymes and study of the chemical nature of the purified preparations, with reference to qualitative reactions, quantitative elementary composition, and the kinds and amounts of amino acid radicles which they contain as determined by the Van Slyke method, the enzyme activity has repeatedly been found to depend upon keeping the protein intact, the activity being lost when the enzyme solution is subjected to treatment which coagulates, hydrolyzes, or otherwise induces chemical change in the protein matter.

Especial mention might be made of the effect of added amino acids in diminishing the rate of hydrolytic inactivation; and of

the quantitative finding that antiseptics which act chemically upon proteins are very much more destructive of the enzyme than are antiseptics of the lipoid-dissolving type. These independent lines of evidence are especially convincing because they link inactivation of the enzyme so closely and definitely with chemical change of the protein matter of the purified enzyme preparation; or, conversely, enzyme activity with intact protein. Figures 8, 9, and 10 illustrate natural enzymes, purified and crystallized by



FIG. 10. — Crystalline trypsin. (Courtesy of Dr. J. H. Northrop.)

Sumner and by Northrop and coworkers. Several other typical enzymes have also been crystallized in other laboratories.

There is now no good reason to doubt, and abundant reason to accept, the conclusion that the best-studied enzymes of the digestive tract are amino acid derivatives of protein or protein-like nature, and that food protein furnishes amino acids to serve as material for body enzymes as well as for body tissue in the more familiar sense. (To avoid even temporary misapprehension, the existence of tissue enzymes and coenzymes containing vitamin constituents may be mentioned here, though the study of them belongs with later chapters.)

• **Other digestive enzymes.** Obviously, the above discussion does not include all enzymes that function in the digestion of food. Others, such as phosphatases, lecithinases, nucleotidases, may be studied in the more detailed literature referred to at the end of this chapter, and also of Chapter VII.

With this brief sketch of the digestive enzymes, the adequate discussion of which would require a volume in itself, we may now pass to a review of the digestive process: the course of the food through the human alimentary tract, and the mechanical and chemical treatment to which it is subjected.

### Salivary and Gastric Digestion

The muscular contractions of the empty stomach which give us the sensation of hunger may be regarded as an indication that the stomach is in a state of readiness to receive food. There is experimental evidence that the stomach digests more expeditiously the food which is "eaten with hunger."

The description of the digestive process which follows presupposes that the food is eaten under favorable conditions and received by a digestive tract which has been permitted to form good and regular habits.

The eating of food induces a flow of saliva from great numbers of minute glands in the lining membrane of the mouth and from the three pairs of large salivary glands. That saliva is secreted in response to psychic as well as chemical stimulation is shown by the fact that actual contact with the food is not necessary, since secretion may be started by the sight or odor or even the thought of food. Mixed human saliva has usually a faintly alkaline reaction and always contains a starch-digesting enzyme (salivary amylase, ptyalin), although its amylolytic activity appears to vary considerably with individuals and with the same individual at different times of the day. As the food comes in contact with saliva, the digestion of starch and dextrin under the influence of the salivary amylase begins at once; but, as mastication is an entirely voluntary act, the thoroughness with which the food becomes mixed with saliva is subject to wide variations.

Usually the food stays too short a time in the mouth for the starch to be acted upon *there* to any great extent, and formerly it was supposed that salivary digestion must cease almost as soon as the food reaches the stomach because the acidity of the gastric



juice is unfavorable to the action of the amylase. The view has now changed as the result of a number of investigations, among which those of Cannon and of Carlson are of especial interest.

When a small amount of an inert metallic compound such as bismuth subnitrate is mixed with the food, it becomes possible to photograph the food-mass within the body by means of Roentgen rays (X-rays). By the use of this method Cannon carried out an extended series of observations upon the movements of the stomach and intestines during digestion, upon the results of which the statements concerning the mechanism of digestion in this chapter are chiefly based.

It is important to distinguish between (a) the elastic and relatively inactive *cardiac* region of the stomach, and (b) its muscular *pyloric* region — the part nearer the outlet to the small intestine.

Cannon's observations, confirmed by those of other investigators, show that the vigorous muscular movements described by Beaumont, which generally begin 20 to 30 minutes after the beginning of a meal, occur only in the middle and pyloric portion of the

stomach, while the cardiac region or fundus, which serves as a reservoir for the greater portion of the food as it enters the stomach, is not actively concerned in these movements and does not rapidly mix its contents with the gastric juice.

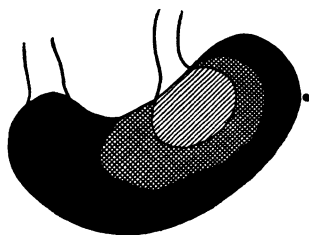


FIG. 11. — Section of frozen stomach of rat during digestion to show the stratification of food given in three successive portions differently colored. (Reproduced from Howell's *Textbook of Physiology*, by permission of the W. B. Saunders Company.)

That there is no general circulation and mixing of the entire stomach contents during or immediately following a meal is further shown by the experiments of Grützner, who fed rats with foods of different colors and, on killing and freezing the animals and examining the stomach contents, found that the portions which had been eaten suc-

cessively were arranged in definite strata. The food which had been first eaten lay next to the walls of the stomach and filled the pyloric region, while the succeeding portions were arranged regularly in the interior in a concentric fashion (Fig. 11). In describing this result Howell says: "Such an arrangement of the food is more readily understood when one recalls that the

stomach has never any empty space within; its cavity is only as large as its contents, so that the first portion of food eaten entirely fills it, and successive portions find the wall layer occupied and are therefore received into the interior."

The character of the gastric juice secreted in different parts of the stomach varies considerably, especially as regards its acidity. In the middle region the secretion is rich in acid, while at both the cardiac and the pyloric ends, the *parietal cells* (formerly called the *border cells* or *cover cells*), from which the secretion of the acid appears to take place, are few in number or entirely lacking, and the juice secreted in these regions may be neutral or, according to Howell, even slightly alkaline.

The nature and extent of the muscular movements also vary greatly in the different regions of the stomach. The peristaltic waves of muscular constriction which bring about the thorough mixing of the food with the gastric juice begin in the middle region and travel toward the pylorus.

The food in the cardiac end of the stomach is not much moved by peristalsis, and so comes only slowly into contact with the gastric juice; and since the juice secreted here contains little if any free acid, a large part of the food mass remains for some time (variously estimated at from 30 minutes to 2 hours or more) in approximately the same neutral or faintly alkaline condition in which it was swallowed, and salivary digestion continues in this part of the stomach without interruption. Thus, if the food has been thoroughly chewed and well mixed with saliva before swallowing, much if not most of its starch may be converted into dextrin and maltose in the cardiac region of the stomach before the activity of the ptyalin is stopped by contact with the acid of the gastric juice.

The fundus, however, is not entirely inactive; rather it functions as a sort of elastic pouch which is distended by, and slowly contracts upon, the food mass, thus gradually tending to move it (and particularly its more fluid portions) into the pyloric region. At intervals the pylorus opens and permits passage of chyme (the fluid or semi-fluid mixture of food material mixed with, and partly digested by, the saliva and the gastric juice) into the small intestine. (If fuller discussion on this point is desired, see Murlin's review listed among references at the end of the chapter.)

As digestion proceeds, the pylorus opens more frequently and

the stomach tends to empty itself more and more freely, until finally the pylorus may open to allow the passage of particles which have not been acted upon by the gastric juice.

Whether the stomach will thus completely empty itself of one meal before the eating of the next will depend both upon the length of the interval and the amount and character of the food which was eaten. Small test meals may disappear in from 1 to 4 hours, but meals approximating one third of the day's food may not disappear entirely from the stomach during 6 or 7 hours.

Ordinarily, when each is fed separately, protein food stays longer in the stomach than carbohydrate; fat longer than protein. Mixtures of fat and protein leave the stomach more slowly than either when fed alone. In general the softer or more fluid the fat the more rapidly it will leave the stomach; also emulsified fats tend to pass on more promptly than fat of the same kind taken in larger masses.

The most important constituents of gastric juice are free hydrochloric acid and pepsin. While other acids may be found in stomach contents, the acidity of gastric juice appears to be due almost entirely to hydrochloric acid, which Carlson has found to constitute an average of 0.40 to 0.50 per cent of normal human gastric juice as secreted. The acid has an antiseptic action on the stomach contents. When through any cause the acidity of the gastric juice is abnormally decreased, the numbers of bacteria in the stomach contents may increase greatly. Also the acidity of the chyme as it passes the pylorus has an important influence upon the secretion of the pancreatic juice.

Carlson distinguishes between three types of normal gastric secretion. There is a slight but continuous secretion of juice in the empty stomach. The flow is augmented by both psychical and chemical stimulation. The psychic secretion (appetite juice) is brought about to some extent by the sight, odor, or memory of food but principally by the acts of tasting and chewing it. Since the continuous secretion is sufficient to initiate gastric digestion, the psychic secretion is not indispensable; and at best it usually ceases within 15 to 20 minutes after the completion of mastication. The chemical stimulation arising within the stomach itself provides for the continuance of gastric digestion, and is more important than either the "continuous" or the "psychic" secretion. This third type of secretion is brought about by the presence of

food in the stomach and also by the stimulating effect of certain substances, including water and dilute acids, which act through some local secretory mechanism in the walls of the stomach and duodenum.

Under normal conditions, the amount of nutritive material absorbed from the stomach is insignificant as compared with the amount absorbed from the intestine. Most of the food eaten is passed from the stomach into the intestine in the form of chyme, having been more or less liquefied and acidulated by its thorough mixing with the gastric juice in the middle and pyloric regions of the stomach.

The stomach therefore has several functions. It serves (1) as a storage reservoir receiving food in relatively large quantities, say three times a day, and passing it on to the intestine in small portions at frequent intervals, (2) as a place for the continuation of the salivary digestion of starch, and (3) for the beginning of the digestion of proteins and perhaps fats, and finally (4) as a disinfecting station of somewhat doubtful and variable value since the food is subjected to the acidity of the gastric juice for a relatively short time in the pyloric region, and the degree of contact of acid with bacteria must depend largely upon the size of the food particles at this stage of digestion.

### Intestinal Digestion

*Digestion in the small intestine.* When the pylorus opens, food, now reduced to liquid chyme, is projected into the upper part of the small intestine, where it usually lies for some time in the curve of the duodenum, until several additions have been made to it from the stomach. While the food rests here the bile and pancreatic juice are poured out upon it, and here also, as well as in other parts of the small intestine, a certain amount of intestinal digestive juice is secreted by the glands of the lining membrane and mixed with the intestinal contents. While for purposes of description the pancreatic and intestinal juices and the bile may be discussed separately, it is to be remembered that in normal digestion they always act together. Cannon's observations showed that after a certain amount of food and digestive juices has accumulated as just described in the first loop of the small intestine, the mass all at once becomes segmented by constrictions of the intestinal walls, and the segmentation is repeated rhythmically

for several minutes, so that the individual portions are subjected to relatively extensive and energetic to-and-fro movement, which is doubtless very important in facilitating the emulsification of fat. Other effects of the muscular constrictions which cause the segmentation are (1) a further mixing of food and digestive juices, (2) the bringing of the digested food into contact with the absorbing membrane, (3) the emptying of the venous and lymphatic vesicles in the membrane, the material which they have absorbed being forced into the veins and lymph vessels by the compression of the intestinal wall.

The fluid food mass which the stomach pours into the duodenum normally contains a small amount of free hydrochloric acid besides a larger amount combined with protein. The pylorus having closed, the bile, the pancreatic juice, and the intestinal juice (all being alkaline) act together to neutralize the acids present.

In man the main duct of the pancreas and the bile duct unite and empty into the small intestine about 8 to 10 cm. (3 to 4 inches) below the pylorus. *The pancreatic juice* is a clear, slightly alkaline liquid containing (or furnishing through their zymogens) not less than three enzymes which play important parts in the digestion of proteins, fats, and carbohydrates respectively.

The outflow of the pancreatic juice begins at once when any of the acid stomach contents passes through the pylorus, and has been shown by Bayliss and Starling to be due to a definite chemical substance, *secretin*, a hormone produced as the result of the action of the acid upon some constituent of the intestinal mucous membrane. Secretin is absorbed and carried by the blood to the pancreas and there stimulates the flows of pancreatic juice.

*The bile* in the intestinal contents greatly increases the solubility of the fatty acids, while at the same time it diminishes the surface tension between watery and oily fluids, thus breaking up the oil droplets and permitting the lipase of the digestive juice to come into more effective contact with the fat of the food. But more importantly it has been shown that bile acids greatly facilitate the absorption of the fatty acids liberated in the digestion of fat. (A review by Verzar, 1933, is cited among the references at the end of the chapter.) This behavior of the bile acids is explained by *in vitro* studies in which it was demonstrated that taurocholic and glycocholic acids possess the ability to bring into aqueous solution fatty acids, and, to a less marked extent, neutral fat.

According to Starling, the rapid flow of bile during intestinal digestion is due not only to the pouring out of what was previously stored in the gall bladder, but also to an increased rate of secretion to which the liver is stimulated by the same chemical mechanism which stimulates the flow of pancreatic juice.

*The intestinal juice* contains at least five enzymes: enterokinase, by the action of which trypsinogen is converted into trypsin; erepsin, which produces further cleavage of the proteoses and peptones into amino acids; and the three enzymes, sucrase (or invertase), maltase, and lactase,\* which hydrolyze respectively the three disaccharides, sucrose, maltose, and lactose.

The acidity of the chyme is, of course, neutralized by the alkalinity of the pancreatic juice, the intestinal juice, and the bile. Without attempting any technical discussion of the matter it may be said that in general under normal conditions the range of hydrogen ion activity found in the intestinal contents is favorable to the activity of the pancreatic and intestinal enzymes. Under such conditions all three classes of foodstuffs are readily attacked by the digestive enzymes present, and brought into condition for absorption — the carbohydrates as monosaccharides; the fats as fatty acids and glycerol; the proteins as amino acids.

Absorption of many digestion products takes place very readily in the small intestine — more readily and completely than can be explained by the purely mechanical laws of diffusion. On this account the process is sometimes called *resorption* to distinguish it from passive absorption such as takes place by diffusion through non-living membrane.

Observations have been made upon a patient having a fistula at the end of the small intestine. In this case it was found that 85 per cent of the protein matter of the food was absorbed before this point was reached, and the absorption of the other foodstuffs is probably equally complete. For this patient the food began to pass the ileocaecal valve in about 2 to 5 hours after eating, but the time required from the eating of the food until the last portions had passed into the large intestine was 9 to 23 hours.

*Digestion in the large intestine.* We have seen that in the small intestine the conditions are very favorable both for digestion and for absorption, and that very much the greater part of the available nutrients has been absorbed before the food mass reaches the ileocaecal valve.

With the passage of material from the ileum into the caecum, the caecum and ascending colon become gradually filled. Observations show that this passive filling takes place very slowly except during and immediately after meals (Hurst). The material remains in the large intestine for a comparatively long time (generally 18 hours or longer). During this time there is a marked absorption of water, along with the remaining products of digestion. The residual material gradually becomes more solid and takes on the character of feces.

### Bacterial Action in the Digestive Tract

The digestive tract of an infant contains no bacteria at birth, but usually some gain access during the first day of life. In the average adult it is estimated that each day's food in its passage through the digestive tract is subjected to the action of over one hundred billion bacteria, chiefly in the large intestine.

Since bacteria are regularly present in the digestive tract in such large numbers, it has been questioned whether they may not perform some essential function in connection with the normal processes of digestion.

If it were possible to exclude absolutely all bacteria from the digestive tract, the well-being of the body would probably not be impaired; yet under such conditions as ordinarily exist, the bacteria which usually predominate in the digestive tract of the thoroughly healthy man probably render an important service in helping to protect against noxious species. *Lactobacillus acidophilus* has come to be regarded as the species best adapted to the function of maintaining a favorable condition in the human intestine. The taking of cultures of this organism and the liberal consumption of lactose and dextrin, the carbohydrates most favorable to it, may assist in the establishment and maintenance of a good condition of intestinal hygiene.

Mitchell and Hamilton (1929) say: "It is a matter of first importance in practical nutrition to determine the effect of different foods and classes of foods on the intestinal flora." They then discuss favorably the method of Bergeim which measures the relative effects of different foods upon the intestinal condition, and conclude that milk, fruit, and vegetables, as well as lactose and dextrin, tend to result in a superior intestinal hygiene; and that the value of the milk is not due simply to its lactose, for casein

gave better results than meat or egg protein or even than the vegetable proteins which were investigated.

### **Coefficients of Digestibility of Food**

The fecal matter passed per day varies considerably in health, but on an ordinary mixed diet of reasonably digestible food materials is usually between 100 and 200 grams of moist substance containing 25 to 50 grams of solids. The feces contain any indigestible substances swallowed with the food and any undigested residues of true food material; but ordinarily they appear to be largely composed of residues of the digestive juices, together with certain substances which have been formed in metabolism and excreted by way of the intestine, and bacteria, living and dead.

Prausnitz studied the feces of several persons placed alternately on meat and on rice diets and found that, although the solids of the meat were about ten times as rich in nitrogen as the solids of the rice, the two diets yielded feces whose solids were of practically the same composition.

In view of such results Prausnitz considers that "normal" feces have essentially the same composition irrespective of the food, the quantity of food residues in such normal feces being negligible. From this point of view the feces show not so much the extent to which the food has been absorbed as whether it is a large or a small feces-former. On the other hand, so far as the nitrogen compounds of the feces are concerned, it is probably true, as generally assumed, that they represent material either lost or expended in the work of digestion, and therefore that the nitrogen of the feces is to be deducted from that of the food in estimating the amount available for actual tissue metabolism. This, however, is by no means equally true of the mineral elements, many of which, after being metabolized in the body, are eliminated mainly by way of the intestine rather than through the kidneys.

On a liberal diet consisting entirely of non-nitrogenous food the amount of nitrogen in the feces was 0.5 to 0.9 gram per day, which is more than is sometimes found in feces from food furnishing enough protein to meet all the needs of the body. Thus the expenditure of nitrogenous material in the digestion of fats and carbohydrates may be larger than in the digestion of protein food.

The feces always contain ether-soluble substances as well as



protein. Fasting men have eliminated 0.6 to 1.3 grams per day of total lipids, a large part of which was sterols.

When the diet is very poor in fat and other lipids, the feces may contain as much as was contained in the food. As the fat content of the food rises, the actual amounts in the feces increase, but the relative amounts decrease, so that up to a certain point the apparent percentage utilization of the fat becomes higher. The limit to the amount of fat which can be thus well digested varies with the individual and with the form in which the fat is given. Quantities up to 200 grams per day have been absorbed to within 2 to 3 per cent when given in the form of milk, cheese, or butter.

In addition to protein and fat the feces always contain various other forms of organic matter which in the routine proximate analyses usually made in connection with feeding experiments are collectively reported as "carbohydrates determined by difference."

With these facts in mind one may make use of the coefficients of digestibility without being misled by them. These coefficients show the relation between the constituents of the food consumed and the corresponding constituents of the feces. Thus if the feces from a given diet contain 5 per cent as much protein as was contained in the food, this proportion is assumed to have been lost or expended in digestion, and the coefficient of digestibility of the protein of the diet is stated to be 95 per cent. While, as just shown, this assumption is not entirely correct, yet it is approximately true of the organic nutrients that the difference between the amounts in the food and in the feces represents what is available to the tissues of the body, and thus these coefficients serve a useful purpose in the computation of the nutritive values of foods.

From the results of hundreds of digestion experiments Atwater computed the average coefficients of digestibility of the organic nutrients of the main groups of food materials, when used by man as part of a mixed diet, to be as shown in Table 7.

In some cases these figures are higher than have been reported for similar foods by other observers, the differences being due mainly to the fact (not formerly recognized) that a food may be more perfectly utilized when fed as part of a simple mixed diet than when fed alone. Milk is an example of such a food, and has, when consumed as part of a mixed diet, a much higher coefficient

of digestibility than is often assigned to it on the basis of earlier experiments.

TABLE 7. — AVERAGE COEFFICIENTS OF DIGESTIBILITY OF FOODS  
WHEN USED IN MIXED DIET (Atwater)

|   | PROTEIN         | FAT             | CARBOHYDRATES   |
|---|-----------------|-----------------|-----------------|
|   | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| Animal foods . . . . .                        | 97              | 95              | 98              |
| Cereals and breadstuffs . . .                 | 85              | 90              | 98              |
| Dried legumes . . . . .                       | 78              | 90              | 97              |
| Vegetables . . . . .                          | 83              | 90              | 95              |
| Fruits . . . . .                              | 85              | 90              | 90              |
| Total food of average mixed<br>diet . . . . . | 92              | 95              | 98              |

It will be seen that the coefficients differ less for the different types of food than might be expected from popular impressions of "digestibility" and "indigestibility." It is also noteworthy that the coefficients of digestibility are less influenced by the conditions under which the food is eaten and vary less with individuals than is generally supposed. In explanation of this it may be said that general impressions of digestibility relate mainly to ease or comfort of digestion, which often bears little relation to the extent to which the food is ultimately digested in its passage through the entire digestive tract.

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## CHAPTER VII

### THE FATE OF THE FOODSTUFFS IN METABOLISM

HOPKINS has well said, "Among the most fundamental of the dynamic chemical events related to life are the oxidations which yield energy to the cell." Between the digestive hydrolyses of the original organic foodstuffs and the actual oxidations there intervene not only the transportation of the nutrients but also chemical changes collectively referred to as constituting the intermediary metabolism. Our knowledge of these intermediary chemical reactions is still tentative notwithstanding much intricate research. In the preface to their excellent book on the carbohydrates the Armstrongs explain that they omit the discussion of carbohydrate metabolism because the subject is "highly controversial and constantly being upset." These reasons appear equally cogent in the writing of the present book. The conciseness essential to its main purpose forbids any attempt to follow the intricacies of the intermediary metabolism in such manner as is done in several of the works referred to at the end of the chapter. We may, however, outline the fate of carbohydrate, of fat, and of the proteins and their amino acids sufficiently to show some important relationships among them.

#### CARBOHYDRATE

The carbohydrate of the food, having been brought to the form of monosaccharide in the intestine, is taken up through the intestinal wall and passes thence to the circulation. Galactose, glucose, and fructose (mentioned in the order of rapidity of absorption) are taken up more readily than if the process were one of simple diffusion, the difference being sometimes indicated by the use of the term resorption; or these three sugars are sometimes said to be "selectively absorbed." This now finds a tentative chemical explanation in the theory that their absorption is accelerated by the combination of these sugars with phosphoric acid (sometimes called phosphorylation) to form compounds which are quickly absorbed.

During rapid absorption of glucose it may reach a concentration of as much as 0.2 per cent in the portal blood; but the blood of the general circulation remains more nearly constant, usually containing a little less than 0.1 per cent of glucose. Thus it appears that a considerable part of the carbohydrate absorbed must be stored temporarily in the liver and given up gradually to the blood in the form of glucose, keeping nearly constant the glucose content of the blood of the general circulation. The carbohydrate thus stored in the liver cells is deposited in the form of glycogen, which, after abundant carbohydrate feeding, may reach 10 per cent of the weight of the liver (or, in rare cases, an even higher figure) and may fall to nearly nothing when no carbohydrate food has been taken for some time. The muscles also store glycogen, their glycogen contents varying from traces to about 2 per cent.

The fact that the carbohydrate stored in the liver after a meal is largely converted into glucose and passes into the blood before the next meal, while the glucose content of the blood remains small and nearly constant, indicates that the glucose of the blood must be rather rapidly used, and from our present standpoint the outstanding question of the carbohydrate metabolism is the fate of the glucose carried to the muscles and other tissues by the blood.

The immediate fate of the glucose is influenced by the balance between the functions of two hormones: *insulin* secreted by the "islet cells" (islands of Langerhans) of the pancreas, and *epinephrine* (adrenaline, adrenine) secreted by the adrenal glands. The effects of these, and doubtless other hormones, are more or less interrelated: we can here consider only their immediate action upon the carbohydrate metabolism. Shaffer and Ronzoni (1932) consider that insulin accelerates (a) conversion of glucose to glycogen and (b) carbohydrate oxidation in liver, muscles, and probably all tissues; and that epinephrine also has two chief effects, (a) increase in the rate of hydrolysis of liver glycogen to glucose, an effect which insulin tends to counteract, and (b) increase in the conversion of muscle glycogen to hexose phosphate and perhaps further to some of the intermediates of the process of breakdown and oxidation outlined below.

### Breakdown and Oxidation of Carbohydrate

It is quite probable that the sequence of chemical changes involved in the intermediary metabolism of carbohydrate, and

the oxidation of the intermediates, may be different at different times and places in the body; but the outstanding feature is the breaking of the hexose (or hexose phosphate or diphosphate) into smaller molecules containing three carbon atoms each. Prominent among the compounds considered as possible intermediates at this stage are: glyceric aldehyde, lactic acid, methylglyoxal (pyruvic aldehyde), pyruvic acid, dihydroxyacetone.

It is largely the interrelationships of these three-carbon compounds, and the relative prominence of one or another of them, on which many differences of view have been expressed by those who have worked and written in this field. An adequate critical analysis of the evidence would require more space than is here available. References may be found at the end of the chapter.

The student will do well to keep clearly in mind that conditions vary sufficiently within the body so that more than one theory of carbohydrate metabolism may be valid in its proper place. The three-carbon compounds just mentioned (and possibly others) are largely interconvertible, both among themselves and with some of the intermediates of the metabolism of fats and proteins.

Moreover, most of them are on the same energy plane, as regards oxidation, with each other and with glucose so that they are easily reconvertible into glucose under appropriate conditions; in fact, to a large extent may be regarded as in dynamic equilibrium with it, and (either through it or directly) with glycogen also.

Pyruvic acid may be regarded as an intermediate oxidation product, or as a product along with glycerol of a Cannizzaro rearrangement of two molecules of glyceric aldehyde, formed from one molecule of hexose or hexose phosphate or diphosphate. The conversion of both pyruvic acid and glycerol into glucose may be demonstrated in the diabetic organism; also that of those intermediates — glyceric aldehyde, dihydroxyacetone, lactic acid — which have exactly the same elementary composition as glucose.

A comprehensive and flexible grasp of the interconvertibility of the several substances which are or may be involved, in a dynamic equilibrium which may shift with circumstances, is probably the best "key" to the present confused literature of intermediary metabolism.

In general, the rate of oxidation (energy metabolism) in the tissues depends more largely upon the activity of the cells than



upon the supply either of oxidizable matter or of oxygen. When a sufficient supply of oxygen is provided, any further increase has little effect upon the rate of combustion, and a surplus of carbohydrate instead of being burned is stored as glycogen, or converted into fat. But, while the absorption of an abundance of carbohydrate does not greatly change the rate of oxidation in the body, it may result in the use of carbohydrate as fuel almost to the exclusion of fat for the time being, as is shown by observations upon the **respiratory quotient**, which is the quotient obtained by dividing the volume of carbon dioxide given off in respiration by the volume of oxygen consumed:

$$\frac{\text{Volume of CO}_2 \text{ produced}}{\text{Volume of O}_2 \text{ consumed}} = \text{Respiratory quotient (R. Q.)}$$

The numerical value of this quotient will evidently depend upon the elementary composition of the materials burned. Carbohydrates will yield a quotient of 1.0, since they contain hydrogen and oxygen in proportions to form water, so that all oxygen used to burn carbohydrates goes to the making of carbon dioxide, and each molecule of O<sub>2</sub> so consumed will yield one molecule of CO<sub>2</sub>, occupying (under the same conditions of temperature and pressure) the same volume as the oxygen consumed to produce it.

Fats contain much more hydrogen than can be converted into water by the oxygen present in the molecule, and therefore a part of the oxygen used to burn fat goes to form water, so that the volume of oxygen consumed is greater than the volume of carbon dioxide produced. The common fats of the body and of the food give quotients approximating 0.7.

Proteins give quotients intermediate between those of carbohydrates and fats, but if the amount of protein used in the body be determined or estimated and allowed for, one may then deduce from the respiratory quotient the proportions of carbohydrates and fats which are being burned in the body at any given time. If, now, the respiratory quotient rises soon after the eating of carbohydrate food, it is evident that the carbohydrate is being used more freely and fat less freely (either absolutely or relatively) than before.

But it is not to be assumed that *every* change of respiratory quotient is due *solely* to a change in the proportions of materials being burned. For example, during muscular exercise the quotient

may rise as the result of a displacement of carbonic acid from the blood by lactic acid, whether or not there is any change in the relative proportions of fat and carbohydrate undergoing oxidation.

### **Storage of Carbohydrate in the Body**

It has been pointed out that, when carbohydrate is absorbed in larger quantity than is required to meet the body's immediate needs for fuel, the surplus normally accumulates as glycogen, which is stored in the liver and the muscles. The amount of carbohydrate which will be stored in the entire body after rest and liberal feeding is estimated at 300 to 400 grams. Thus the total amount of carbohydrate which can be stored as such in the body is no more than is frequently taken in one day's food. When the body has no tendency to increase its store of glycogen, the further surplus of carbohydrates tends to be converted into fat.

### **Production of Fat from Carbohydrate**

Experimental evidence of the transformation of carbohydrate into fat has been cited in Chapter III, where it was shown that animals which fatten readily on carbohydrate food may store more body fat than could possibly be derived from the fats and proteins eaten; that milch cows have yielded more fat in the milk than could be accounted for on any other view than that fat was formed from carbohydrate; and that there may be more carbon stored in the body from the carbohydrate food eaten by a fattening animal than can be explained in any other way than that a part of the carbon taken into the body as carbohydrate was retained as body fat.

Further proof of the ability of the animal body to change carbohydrate into fat is obtained from the respiratory quotient. As noted above, observations made after a fast tend to show quotients approaching that of fat, while after feeding carbohydrates the quotient may rise rapidly. If the quotient reaches 1.0 it shows that the body as a whole is using carbohydrate and not fat as fuel; and a quotient greater than 1.0 may be taken as evidence that the carbohydrate is itself supplying part of the oxygen which appears as carbon dioxide, or, in other words, that it is breaking down in such a way that a part is burned while another part goes to form in the body a substance more highly carbonaceous and having a lower respiratory quotient than the

carbohydrate itself. In many cases it is certain that this substance must be mainly if not entirely fat. Respiratory quotients greater than 1.0 have been observed after liberal carbohydrate feeding in several species, including man. Each such observation furnishes evidence of a conversion of carbohydrate into fat.

The formation of fat from carbohydrate in the animal body is therefore established by four distinct lines of experimental evidence: (1) by determination of the amounts of body fat formed, (2) by determination of the milk fat produced, (3) by observation of the amount of carbon stored, (4) by observations upon the respiratory quotient.

According to the Coris, there are considerable individual differences in the capacity of the body to store carbohydrate as glycogen. There also appear to be individual differences in capacity for fattening. Yet there can be few if any exceptions to the general relationship that the amount of surplus carbohydrate fuel which the body can store as carbohydrate is far exceeded by the further amount which it can store by conversion into body fat. The normal person probably can, if he wishes, gradually accumulate "stored calories" in the form of body fat up to something like one hundred times the number of calories that he can store directly as carbohydrate. •

### Chemical Steps in the Formation of Fat from Carbohydrate

While there is no doubt whatever of the ability of the animal to synthesize fat from carbohydrate, the mechanism of the process is far from clear. Leathes, according to whom "the chemical changes involved are fascinating in their obscurity," argues that acetaldehyde molecules formed in the course of carbohydrate catabolism can undergo *aldol condensation* not only two at a time but also in larger numbers to form the fatty acids of natural fats.

Smedley developed an alternative hypothesis regarding the mechanism of fatty acid synthesis from carbohydrate material according to which the most probable starting point is a condensation of pyruvic acid with acetaldehyde followed by loss of  $\text{CO}_2$  and molecular rearrangement.

Each of the above hypotheses assumes as a starting point only substances which we have good reason to believe are regularly formed in carbohydrate metabolism, and both are consistent with the well-known fact that natural fats contain fatty acid radicals

having all multiples of two carbon atoms from four to eighteen, but probably none having uneven numbers of carbon atoms in the molecule.

While stearic, palmitic, and oleic acids all appear to be readily synthesized in the animal body from carbohydrate, it appears that linoleic and/or linolenic (or some isomeric or analogous) acid must be derived from the food. Bloor, in 1932, added, "except possibly in the case of young animals"; but this latter suggestion may have arisen from lack of the evidence, since supplied, that milk fat contains linoleic acid.

### FAT

In digestion, the fats are split into fatty acids and glycerol which, however, upon absorption are recombined into neutral fat. It is believed that this recombination occurs during the passage of these digestion products through the intestinal wall. The fat thus absorbed is taken up chiefly if not entirely by the lymph vessels, and is poured with the lymph into the blood.

After a meal rich in fat the chylomicrons (tiny fat particles) may enter the blood abundantly; but most of this fat is rapidly distributed by the blood to the tissues. Bodansky states that when a person at rest eats one gram of fat per kilo of body weight, the concentration of fat in the blood reaches a maximum in four hours and returns to normal in six to seven hours. In general, recent work indicates that the fat content of human blood is more constant, and hence that the fat we absorb must leave our blood more rapidly, than we formerly supposed.

The fat thus leaving the blood may be burned as fuel, or stored for use as fuel in the future. When burned, its energy is used for essentially the same functions as is that of carbohydrate. The average results of a long series of very thorough experiments by Atwater and associates indicated that when both were fed as constituents of a normal mixed diet, the potential energy of fat was about 95 per cent as efficient as that of carbohydrate for the production of muscular work; while the corresponding estimate more recently reached by Krogh and Lindhard is 89 per cent.

### Oxidation of Fat in the Body: $\beta$ -oxidation Theory

*The glycerol* from fat is presumably oxidized first to glyceric aldehyde or some related 3-carbon compound whose fate is then

doubtless the same as when the same substance is formed in carbohydrate metabolism.

*The fatty acid* presents a separate problem and it is this that one has in mind in speaking of the metabolism of fat. The  $\beta$ -oxidation theory is now generally accepted; but this does not exclude the possibility that there may be other paths of metabolism as well. Dakin (1928) remarks, "While Knoop's theory of the  $\beta$ -oxidation of normal fatty acids is based on such a variety of evidence that its general truth can hardly be questioned, there is of course no good reason for assuming that initial oxidation of hydrogen in the  $\beta$ -position is the only type of initial transformation of saturated normal fatty acids that the body is capable of effecting." And Bloor (1933) states explicitly: "That  $\beta$ -oxidation is not the only method of oxidation of the fatty acids is evident from the work of Raper and Wayne . . . and . . . of Quick." According to the  $\beta$ -oxidation theory, the fatty acids are attacked by oxidation at the  $\beta$ -carbon atom with the probable formation first of  $\beta$ -hydroxy, and then of  $\beta$ -ketonic acids. Further oxidation at this point must then cause a separation of the  $\alpha$ - and  $\beta$ -carbon atoms; thus two carbons of the original fatty acid break away, presumably to undergo complete oxidation, and there remains a fatty acid with two less carbon atoms than the original. By such a process, stearic acid would yield palmitic; palmitic would yield myristic; myristic, lauric; and so on to butyric acid. Beta-oxidation of butyric acid would yield  $\beta$ -hydroxybutyric acid and acetoacetic acid. Normally the acetoacetic acid should yield two molecules of acetic which in turn should burn to carbon dioxide and water.

It has, however, been found that in diabetes or starvation, or on a diet containing too much fat and too little carbohydrate, the oxidation of acetoacetic acid as represented in the above scheme is sometimes not complete. More or less acetoacetic acid, and also acetone derived from it, may accumulate in the body and appear in the excretions. This is called a condition of ketosis, and is now explained on the basis of the view that the normal catabolism of acetoacetic acid takes place only in the presence of a simultaneous catabolism of carbohydrate, or of substances such as are formed in the intermediary metabolism of carbohydrate (and also of some amino acids). This bringing about of the normal completion of the  $\beta$ -oxidation process, thus preventing ketosis, is called antiketogenic action.

Because of their tendency to form "acetone bodies" \* under certain conditions, the fatty acids are said to have ketogenic properties. Since certain amino acids, leucine, phenylalanine, and tyrosine, have also been found to be a potential source of "acetone bodies," proteins are likewise ketogenic to the degree that these amino acids are present in the protein molecule. On the other hand, some of the amino acids are, like the carbohydrates, "antiketogenic"; and the glycerol from fat may doubtless be converted to glucose (or equivalent intermediary products) in the course of metabolism. Consequently both fat and protein yield antiketogenic as well as ketogenic derivatives. The relation between the total number of potential ketogenic molecules from all sources in the diet and the total number of antiketogenic molecules is known as the ketogenic-antiketogenic ratio  $\left(\frac{K}{A}\right)$ .

Different investigators of the subject seem to attach very different degrees of importance to this ratio.

### Storage of Food Fat in the Body

That fat derived from the food may be stored as body fat has already been shown (Chapter III) and need not be discussed further here. Mills (1911) found that fatty oils injected with antiseptic precautions into the subcutaneous tissue may, under favorable conditions, be absorbed therefrom and used in the body in the same way as if obtained by feeding; but Koehne and Mendel found further that fatty oils introduced parenterally were too slowly distributed and metabolized to be a dependable source of any considerable proportion of the fuel needed for the support of the energy requirements in nutrition.

As noted in Chapter III, experiments with deuterium fats emphasize the extent to which the absorbed fatty acids go into the body's fat stores before undergoing oxidation.

Whether fat once deposited will remain and accumulate or be returned to the circulation and used as fuel, will depend upon the balance between the food consumption and the food requirements of the organism as a whole. In this respect, there is no known difference between fat consumed and deposited as such and fat formed in the body from other food materials.

\* This is a case in which substances are called "bodies" so persistently that the usage partakes of the nature of an accepted technical term.

### **Can Carbohydrate Be Formed from Fat?**

Glycerol is readily convertible into glucose in the body, probably passing through the form of glyceric aldehyde as an intermediate step; but the glycerol radicle represents only about one twentieth of the energy value of the fat molecule.

Whether carbohydrate is ever formed from fatty acid in the animal body is a question which has been much debated. We shall not attempt to study the conflicting evidence and arguments here, partly for lack of space, partly because it involves more of the pathology of diabetes than belongs within the scope of this book, and partly because fat can be used to so large an extent interchangeably with carbohydrate that whether or not it is literally convertible into carbohydrate is not a question of great importance from the chief viewpoint of our present study.

### **PROTEIN**

It is now believed that the hydrolysis of proteins to amino acids in the digestive tract is, in normal cases, practically complete. The significance of this digestive cleavage lies not simply in the formation of more soluble and more readily diffusible substances, but also in the resolution of the complex molecules of food protein into their simple amino-acid "building stones," which may be very extensively rearranged by the body in the synthesis of its own tissue proteins.

### **Absorption and Distribution of Protein Digestion Products**

The amino acids resulting from digestive hydrolysis of the food proteins pass through the intestinal wall and into the blood and are thus distributed throughout the body. They are rapidly absorbed from the blood into the various tissues. Thus each tissue receives its protein material in the form of amino acids from which can be synthesized the particular kind of protein characteristic of the tissue in question. In other words each tissue makes its own proteins from the amino acids brought by the blood. Amino acids not used in synthesizing protein (whether brought by the blood or liberated from tissue material) are deaminized.

### Utilization of Protein in the Tissues

The proteins of the digested food, absorbed and distributed in the form of amino acids as described above, soon become available for nutrition; and among other functions they, like the carbohydrates and fats, may be oxidized as fuel for muscular work. (It will of course be understood that the protein is not supposed to be oxidized directly. Protein is split into amino acids, the amino acids deaminized, and the non-nitrogenous residues of the amino acids are oxidized.) Pflüger showed that protein may serve as a source of muscular energy by feeding a dog for 7 months exclusively upon meat practically free from fat and carbohydrate, and requiring it throughout the experiment to do considerable amounts of work, the energy for which must in this particular case have been derived largely from the protein consumed.

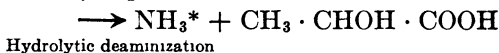
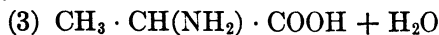
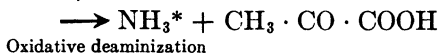
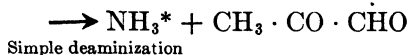
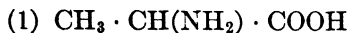
The experimental facts and theoretical explanations regarding the intermediary metabolism of proteins (or of the amino acids arising from them) in the body tissues must now be considered. By experiment it has been found that if a meal extra rich in protein be eaten, an increased elimination of nitrogenous end products can be observed within 2 or 3 hours, and probably much the greater part of the surplus nitrogen will have been excreted within 24 hours of the time it was taken into the stomach. It does not follow, however, that the surplus nitrogen ingested supplies (directly and literally)\* all of the extra nitrogen excreted or that the whole of the protein molecule is broken down and eliminated at the same rate. Evidently, the nitrogenous radicles of the protein may be split off in such a way as to leave a non-nitrogenous residue in the body, and the study of protein metabolism involves a consideration of the fate of both the nitrogenous and the non-nitrogenous derivatives.

### Chemistry of the Deaminization

It is probable that the deaminization may occur, according to conditions existing at the place and time, in any of three ways as illustrated by the following reactions of alanine:

\* In the sense sometimes called "bookkeeping with the body" the extra excretion is entirely *attributable to* the extra intake; but as previously inferred from the concept of dynamic equilibria, and now directly demonstrated in experiments with isotopic nitrogen, there is always some *exchange of material*.





Thus a typical amino acid such as alanine may be deaminated (deaminized) to yield methylglyoxal, pyruvic acid, and lactic acid, respectively. From what we have already seen of the intermediary metabolism of carbohydrate, it is to be expected that these non-nitrogenous derivatives of protein may be changed into carbohydrate in the body, and this expectation is fully realized in the study of the evidence of direct experimentation.

### Formation of Carbohydrate from Protein

As early as 1876 Wolffberg tested the formation of carbohydrate from protein by fasting fowls for two days in order to free them from glycogen and then feeding for two days with meat powder which had been washed free from carbohydrate. Two of the fowls were killed soon after this protein feeding and showed more glycogen in their livers and muscles than could be accounted for except as derived from the protein fed. This formation of glycogen from protein was fully confirmed by Kulz in a long series of experiments in which the food consisted of chopped meat thoroughly extracted with warm water (Lusk). Much additional evidence of the production of carbohydrate from protein could be cited.

The most striking evidence of the formation of carbohydrate from protein in the animal body is found in the many observations and experiments which have been made in cases of diabetes, and in experimental glycosuria produced either by administration of phlorizin or by removal of the pancreas. In such cases large amounts of carbohydrate may be excreted in the form of glucose even when there is little body fat, and no carbohydrate

\* The large amounts of nitrogen split off in this way from amino acids are transported in the blood, not as ammonia but largely as urea. Luck (1933, 1935) discusses the problem of the way in which the transformation of ammonia to urea is accomplished.

is fed. The glucose must therefore result from the metabolism of protein. In Lusk's exhaustive experiments upon dogs rendered diabetic by phlorizin, 58 per cent of the total weight of protein broken down in the body (whether in fasting or on a meat diet) was eliminated in the form of glucose.

### Production of Fat from Protein

At various times there has been much controversy regarding the formation of fat from protein in the animal body. As there is now abundant experimental evidence of the production of carbohydrate from protein and of the transformation of carbohydrate into fat, it is evident that protein food can in one way or another contribute to the formation of fat in the body. "The  $\alpha$ -hydroxy or  $\alpha$ -ketonic acids resulting from the deamination of amino acids may evidently be oxidized directly, or they may be converted into sugar and stored as glycogen, or converted into fats" (Mitchell and Hamilton, 1929).

### The Fate of the Nitrogen in Protein Metabolism

*Urea and ammonia.* In the breaking down of body proteins there is doubtless a liberation of amino acids. Amino acids either from this source or from the food are subject to deaminization in the tissues, and in so far as  $\alpha$ -amino groups are concerned the process doubtless consists chiefly in the splitting out of these amino groups, most of which are converted into urea. In health and on a liberal protein diet from 82 to 88 per cent of the total nitrogen excreted by the kidneys is usually in the form of urea. On a low protein diet this percentage is lower. Normally, about 2 to 6 per cent of the total nitrogen eliminated is in the form of ammonium salts, the amount depending largely upon the relation between the amounts of acid-forming and of base-forming elements in the food, which will be discussed in Chapter XIII.

*Uric acid and the purine bases (nucleic acid metabolism).* A part of the nitrogen of human urine is always in the form of uric acid and purine bases. These owe their origin either to the free purine substances of the food, such as the guanine and hypoxanthine of meat extract, or to the metabolism of nucleic acid derived from the nucleoproteins of the food or of the body tissues. The constituent groups of the nucleic acids, their modes of linkage, and the order of their liberation on hydrolytic cleavage such as

occurs in metabolism have been very fully discussed by Levene and Bass (1931).

Purines undergoing metabolism in the body may be derived either (1) from the nucleoprotein of body tissue or (2) from the food, which may contain both nucleoproteins and free purines. On ordinary mixed diet the total urinary output of uric acid averages about 0.6 to 0.7 gram per man per day. The usual range is about 0.5 to 1.0 gram of uric acid per man per day, in which case the uric acid nitrogen constitutes about 1 to 3 per cent of the total nitrogen of the urine.

*Creatine and creatinine.* Chemically, creatinine is the anhydride of creatine. The biochemical relationships and nutritional significance of these substances have been much discussed and the literature is far too extensive to be summarized satisfactorily here. The subject is fully discussed in the monograph of Hunter (1928). See also Mitchell and Hamilton (1929), Rose (1933, 1935), and Bodansky (1936).

The quantity of creatinine excreted is fairly constant for the individual, averaging about 0.02 gram per kilogram of body weight per day. On ordinary mixed diet the creatinine nitrogen usually constitutes 3 to 7 per cent of the total nitrogen of the urine.

*Distribution of excreted nitrogen as influenced by level of protein metabolism.* The above statements regarding the distribution of the eliminated nitrogen among the different end products refer to results obtained upon an ordinary mixed diet containing an average amount of protein. Folin has shown, by a careful and extended study of the urines of healthy men living first upon high and then upon low protein diets, that the distribution of the nitrogen between urea and the other nitrogenous end products depends

TABLE 8. — NITROGEN DISTRIBUTION IN URINE AS INFLUENCED BY LEVEL OF PROTEIN INTAKE (Folin)

|                          | ON HIGH PROTEIN DIET<br>(Free from Meat) |          | ON LOW PROTEIN DIET<br>(Starch and Cream) |          |
|--------------------------|--|----------|---|----------|
|                          | grams                                    | per cent | grams                                     | per cent |
| Total nitrogen . . . .   | 16.8                                     | —        | 3.6                                       | —        |
| Urea nitrogen . . . .    | 14.7                                     | 87.5     | 2.2                                       | 61.7     |
| Ammonia nitrogen . . .   | 0.49                                     | 2.9      | 0.42                                      | 11.3     |
| Uric acid nitrogen . . . | 0.18                                     | 1.1      | 0.09                                      | 2.5      |
| Creatinine nitrogen . .  | 0.58                                     | 3.6      | 0.60                                      | 17.2     |
| Undetermined nitrogen .  | 0.85                                     | 4.9      | 0.27                                      | 7.3      |

very largely upon the absolute amount of nitrogen metabolized (level of protein metabolism). In the case of a man who on one day consumed high protein diet free from meat, and a week later was living on a diet of starch and cream which furnished in all about 6 grams of protein per day, the distribution of end products was changed as shown in Table 8.

Thus, on passing from the high protein to the low protein diet (both being free from meat products) there was a marked decrease in both the absolute and the relative amounts of urea; and a decrease in the absolute, but increase in the relative, amount of uric acid; while the absolute amount of creatinine remained unchanged, so that its relative amount was greatly increased.

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## CHAPTER VIII

### THE FUEL VALUE OF FOOD AND THE ENERGY REQUIREMENT OF THE BODY

WE HAVE seen that, under normal conditions, carbohydrate after its absorption into the body may either be oxidized, or stored as glycogen, or transformed into fat; that fat may be oxidized or stored and that at least its glyceryl radicle may be converted into carbohydrate; and that protein absorbed as amino acids may either be built up into body protein, or deaminized and oxidized, or may yield carbohydrate, or may (either directly or indirectly) contribute to the production of fat. It has also been shown that any or all of these foodstuffs may be utilized as fuel for muscular work.

Thus the body is not restricted to the use of any one foodstuff for the support of any one kind of work, but on the contrary has very great power to convert one nutrient into, or use it in place of, another, and so to utilize its resources that the total potential energy of all of these nutrients is economically employed to support the work of all parts of the organism. The carbohydrates, fats, and proteins stand in such close mutual relations in their service to the body that for many purposes we may properly consider the food as a whole with reference to the total nutritive requirements, provided a common measure of values and requirements can be found. Since the most conspicuous nutritive requirement is that of energy for the work of the body, and since these organic nutrients all serve as fuel to yield this energy, a common basis of comparison is that of energy value.

The energy value of food is usually and conveniently expressed in terms of heat units (Calories); but it is important to realize that the body is not a heat engine. In a heat engine, heat is the source of the work; in the body the heat is rather the result of the internal and external work which the body does. With this clearly in mind there need be no real misconception in using heat units to express the energy values of foods and the energy requirements of the body.



### Heats of Combustion of the Foodstuffs

The calorific value or heat of combustion of any substance, i.e., the amount of energy liberated (transformed) by the burning of a given quantity of the

combustible material, is best determined by means of the bomb calorimeter devised by Berthelot. The particular form of Berthelot bomb which was most used in the quantitative development of the fundamental energy relations of food materials and physiological products was that of Atwater and Blakeslee, fully described by Atwater and Snell in the *Journal of the American Chemical Society*, for July, 1903. In outline (Fig. 12) it consists of a heavy steel bomb, A, with a platinum or gold-plated copper lining and a cover held tightly in place by means of a strong screw collar. A weighed amount of sample is placed in a capsule, B, within the bomb, which is then closed except for the oxygen valve, charged with oxygen to a pressure of at least 20 atmospheres (300 pounds or

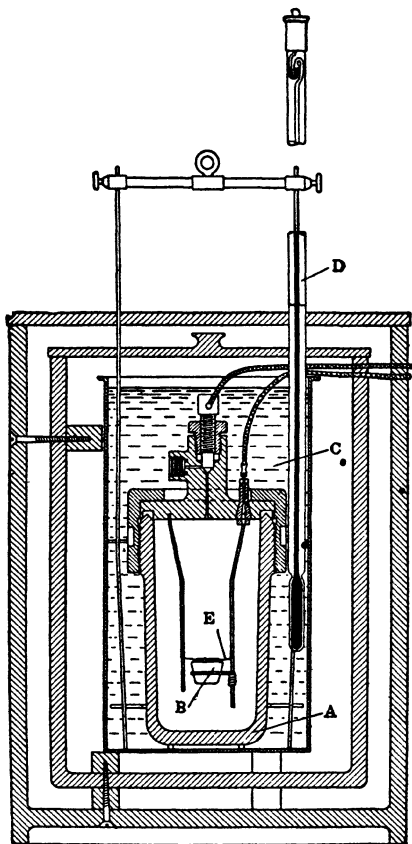


FIG. 12. — The Atwater bomb calorimeter.

more to the square inch), oxygen valve closed, and the bomb immersed in a weighed amount of water, C. The water is constantly stirred and its temperature taken at intervals of one minute by means of a differential thermometer, D, capable of being read to one thousandth of a degree. After the rate at which the temperature of the water rises or falls has been determined, the sample is ignited by means of an electric fuse, E, and, on account of the large amount of

oxygen present, undergoes rapid and complete combustion. The heat liberated is communicated to the water in which the bomb is immersed, and the resulting rise in temperature is accurately determined. The thermometer readings are also continued through an "after period," in order that the "radiation correction" may be calculated and the observed rise of temperature corrected accordingly. This corrected rise, multiplied by the total heat capacity of the apparatus and the water in which it is immersed, shows the total heat liberated in the bomb. From this must be deducted the heat arising from accessory combustions (the oxidation of the iron wire used as a fuse, etc.) to obtain the number of Calories\* arising from the combustion of the sample.

The necessity for corrections for heat loss is avoided in the adiabatic form of the bomb calorimeter which is now more generally used.

Another apparatus for determination of the energy value of foods is the oxy-calorimeter, devised by Benedict and coworkers, which measures the volume of oxygen required to burn a known weight of the food. From this, by means of factors established by use of the bomb calorimeter, the calorific value of the food is calculated. The apparatus, Fig. 13, consists of a combustion chamber, *A*, in which the weighed sample is burned, a soda lime container for absorption of carbon dioxide, *B*, a spirometer for measuring the oxygen used, *S*, and a motor-blower unit for circulating the gas mixture, *C*. The factors for converting liters of oxygen to calories as given by Benedict and Fox in *Industrial and Engineering Chemistry*, Volume 17, page 912 (1925), will be found in Table 60, Appendix A.

The heat of combustion of organic substances is closely connected with their elementary composition. One gram of carbon burned to carbon dioxide yields 8.08 Calories and 1 gram of hydrogen burned to water yields 34.5 Calories. If a compound consisting of carbon and hydrogen only be burned, it gives nearly the amount of heat which these would give if burned separately.

On the other hand, in carbohydrates and fats, which are composed of carbon, hydrogen, and oxygen, the carbon and hydrogen are already partly oxidized by the oxygen present in the molecule; so that 100 grams of glucose, for example, containing

\* When the term Calorie is used in this work it will be understood to mean the large calorie, or kilogram calorie, i.e., the amount of heat required to raise the temperature of one kilogram of water one degree centigrade. This is very nearly the same as the heat required to raise four pounds of water one degree Fahrenheit.

40 grams carbon, 6.7 grams hydrogen, and 53.3 grams oxygen, would yield considerably less heat than would be obtained by burning 40 grams of pure carbon and 6.7 grams of pure hydrogen to carbon dioxide and water respectively.

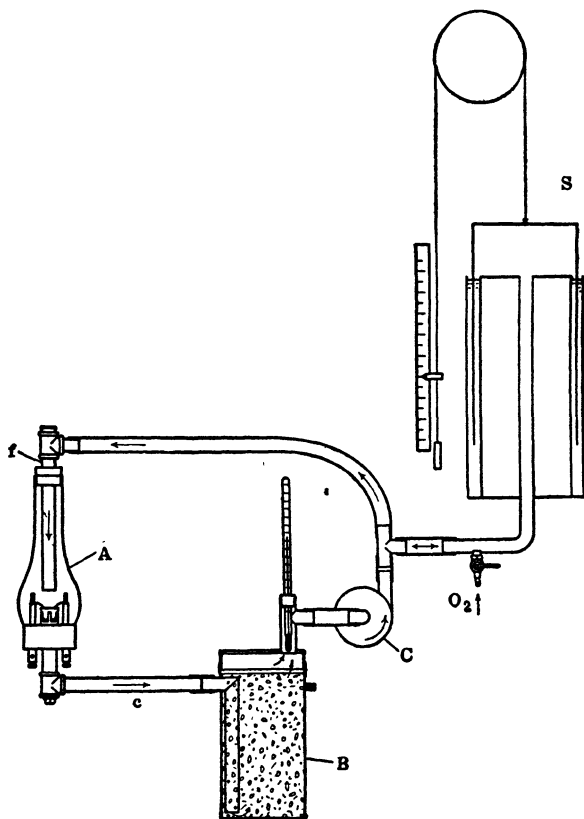


FIG. 13. — Diagram of Benedict oxy-calorimeter. (Courtesy of Dr. F. G. Benedict.)

Proteins when burned in the calorimeter give off their carbon as carbon dioxide, their hydrogen as water, and their nitrogen as nitrogen gas.\* Thus the nitrogen contributes nothing to, and takes nothing from, the heat of combustion; and the latter is essentially dependent here, as in the case of carbohydrates and fats, upon

\* As a matter of fact a small part of the nitrogen is oxidized to nitric acid in the bomb calorimeter, but this is determined and its heat of formation subtracted, so that the final results are as stated above.

the amounts of carbon and hydrogen present and the extent to which they are already combined with oxygen. A little additional heat is obtained by the burning of the small amount of sulfur present in the protein.

The relation between the elementary composition and heat of combustion will be made clearer by the study of Table 9, which includes a number of typical compounds found in the food or formed in the body.

TABLE 9. — HEATS OF COMBUSTION AND APPROXIMATE ELEMENTARY COMPOSITION OF TYPICAL COMPOUNDS

| SUBSTANCE  | HEAT OF COMBUSTION<br>CALORIES<br>PER GRAM | CARBON<br>PER CENT | HYDROGEN<br>PER CENT | OXYGEN<br>PER CENT | NITROGEN<br>PER CENT | SULFUR<br>PER CENT | PHOSPHORUS<br>PER CENT |
|------------|--|--------------------|----------------------|--------------------|----------------------|--------------------|------------------------|
| Glucose .  | 3.75                                       | 40.0               | 6.7                  | 53.3               | —                    | —                  | —                      |
| Sucrose .  | 3.96                                       | 42.1               | 6.4                  | 51.5               | —                    | —                  | —                      |
| Starch     | 4.22                                       | 44.4               | 6.2                  | 49.4               | —                    | —                  | —                      |
| Glycogen } |  |                    |                      |                    |                      |                    |                        |
| Body fat   | 9.60                                       | 76.5               | 12.0                 | 11.5               | —                    | —                  | —                      |
| Butter fat | 9.30                                       | 75.0               | 11.7                 | 13.3               | —                    | —                  | —                      |
| Edestin .  | 5.64                                       | 51.4               | 7.0                  | 22.1               | 18.6                 | 0.9                | —                      |
| Legumin    | 5.62                                       | 51.7               | 7.0                  | 22.9               | 18.0                 | 0.4                | —                      |
| Gliadin .  | 5.74                                       | 52.7               | 6.9                  | 21.7               | 17.7                 | 1.0                | —                      |
| Casein .   | 5.85                                       | 53.1               | 7.0                  | 22.5               | 15.8                 | 0.8                | 0.8                    |
| Albumin    | 5.80                                       | 52.5               | 7.0                  | 23.0               | 16.0                 | 1.5                | —                      |
| Gelatin .  | 5.30                                       | 50.0               | 6.6                  | 24.8               | 18.0                 | 0.6                | —                      |
| Creatinine | 4.58                                       | 42.5               | 6.2                  | 14.1               | 37.2                 | —                  | —                      |
| Urea .     | 2.53                                       | 20.0               | 6.7                  | 26.7               | 46.6                 | —                  | —                      |

Since the energy used in the body is obtained from the oxidation of the same kinds of compounds which exist in food, *i.e.*, from carbohydrates, fats, and proteins (or their cleavage products), we can estimate the amount of energy transformed in the body if we know the amount of each kind of foodstuff oxidized. Account must, however, be taken of the completeness of the oxidation in each case.

When undergoing complete oxidation in the bomb calorimeter, the foodstuffs yield the following average heats of combustion:

|               |      |                     |
|---------------|------|---------------------|
| Carbohydrates | 4.1  | Calories per gram * |
| Fats          | 9.45 | Calories per gram * |
| Proteins      | 5.65 | Calories per gram * |

\* These are weighted averages, taking account of relative amounts of different carbohydrates, different fats, and different proteins in ordinary food supplies.

In the body, carbohydrates and fats are oxidized to the same products as in the calorimeter and so yield the same amounts of heat. Protein, however, which burns in the calorimeter to carbon dioxide, water, and nitrogen, yields in the body no free nitrogen, but urea and other organic nitrogen compounds which are eliminated as end products. These organic nitrogenous end products are combustible; they represent a less complete oxidation of protein in the body than in the bomb calorimeter. The loss of potential energy calculated on the assumption that all nitrogen left the body as urea would be about 0.9 Calorie per gram of protein, but on account of the elimination of other substances of higher heat of combustion (creatinine, uric acid, etc.), the actual loss in the form of combustible end products is considerably greater and averages about 1.3 Calories for each gram of protein broken down in the body.

Hence, when the body burns material which it has previously absorbed, it obtains:

|                              |                        |
|------------------------------|------------------------|
| From carbohydrates           | 4.1 Calories per gram  |
| From fats                    | 9.45 Calories per gram |
| From protein (5.65 - 1.30 =) | 4.35 Calories per gram |

In calculating the fuel value of the food, however, allowance must be made for the fact that a part of each of the materials is lost in digestion, as explained in Chapter VI.

The approximate averages on a mixed diet are:

|               |                       |
|---------------|-----------------------|
| Carbohydrates | 2% lost, 98% absorbed |
| Fats          | 5% lost, 95% absorbed |
| Protein       | 8% lost, 92% absorbed |

The approximate *physiological fuel values* of the food constituents are then:

|               |   |
|---------------|---|
| Carbohydrates | $4.1 \times 98\% = 4.$ Calories per gram  |
| Fats          | $9.45 \times 95\% = 9.$ Calories per gram |
| Protein       | $4.35 \times 92\% = 4.$ Calories per gram |

These physiological fuel values are known as the Atwater and Bryant factors for calculating fuel values of food.

The figures given by Rubner as representing the fuel values of food constituents were as follows:

|               |     |
|---------------|-----|
| Carbohydrates | 4.1 |
| Fats          | 9.3 |
| Protein       | 4.1 |

These were derived from experiments with dogs fed on meat, starch, sugar, etc., and therefore do not allow for so much loss in digestion as has been found to occur with men living on ordinary mixed diet.

### Fuel Value of Food in Nutrition

If the composition of a food is known, its approximate fuel value is easily computed by means of the above factors. Thus milk of about average composition contains:\*

Protein, 3.5 per cent; fat, 3.9 per cent; carbohydrate, 4.9 per cent.

One hundred grams of such milk will furnish in the form of protein,  $(3.5 \times 4. =) 14.0$  Calories; of fat,  $(3.9 \times 9. =) 35.1$  Calories; of carbohydrate,  $(4.9 \times 4. =) 19.6$  Calories; total for 100 grams of milk, 68.7 Calories.

Eggs are estimated to contain, on the average, in the edible portion, 12.8 per cent of protein, 11.5 per cent of fat, and 0.7 per cent of carbohydrate. They would then furnish, per 100 grams, 158 † Calories.

As sources of energy, the quantities of foods to be taken as equivalent or mutually replaceable are those which furnish equal fuel value, *e.g.*, 100-Calorie portions, the weights of which may be calculated directly from the fuel values of 100 grams.

Thus, for milk: 100 grams furnish 69 Calories; then, if  $x$  be the number of grams which furnish 100 Calories:

$$100 : 69 :: x : 100; \quad x = 145.$$

Similarly for eggs:

$$100 : 158 :: x : 100; \quad x = 63.$$

\* In this book, statements of proximate composition and energy values of foods are based on the Federal revised averages (Chatfield and Adams, 1940) as given in Circular No. 549 of the United States Department of Agriculture.

† From here on it will be considered sufficiently accurate to state these quantities to the nearest whole number.

And since the two extremes in the proportion are always the same, the weight in grams of the 100-Calorie portion may always be found by dividing 10,000 (the product of the extremes) by the number of Calories per 100 grams.

The fuel value of foods is often stated in Calories per pound. Thus in the same table from which the above figures for composition are taken, the fuel value of milk is given as 310 Calories per pound. Since 453.6 grams furnish 310 Calories,

$$453.6 : 310 :: x : 100; \quad x = 146,$$

— the number of grams required to furnish 100 Calories. Hundred-Calorie portions of a few typical foods, with the distribution of the Calories between protein, fat, and carbohydrate, are shown in Table 10. In Appendix B the 100-Calorie portions of a much larger number of foods are given, along with their proximate composition and their energy values per 100 grams.

Since proteins and carbohydrates have the same average fuel value and the ash of food does not as a rule constitute a large percentage, the striking differences in the energy values of foods per 100 grams and in the weights of the various foods required to furnish 100 Calories are chiefly referable to differences in water content or fat content or both. That beans have nearly 8 times the fuel value of carrots is essentially due to the difference in moisture, while the difference in fuel value between lean beef and bacon, or between codfish and salmon, is chiefly a matter of fat content. Meat free from fat is about three fourths water and one fourth protein, and so has a fuel value of about one Calorie per gram, while clear fat has a fuel value about nine times as great.

Fuel values of meats as given in the standard tables are apt to be somewhat misleading, inasmuch as they allow for all the fat ordinarily found on the various cuts as taken from the animal, whereas in many cases a considerable part of this fat is trimmed off by the butcher and treated as a by-product; and often much of the remaining fat is removed either in the kitchen or at the table. If a pound of steak consists of 14 ounces of clear lean and 2 ounces of clear fat, and the fat is not eaten, at least half of the total fuel value of the pound of steak is lost.

Many vegetables are more watery than lean meats and so contrast even more strikingly with the fats. An ounce of clear fat pork is equal in fuel value to about two pounds of cabbage;

TABLE 10. — HUNDRED-CALORIE PORTIONS OF TYPICAL FOODS

| Food<br>(Edible Portion)          | WEIGHT OF 100-<br>CALORIE PORTION |        | DISTRIBUTION OF CALORIES |           |                         |
|-----------------------------------|-----------------------------------|--------|--------------------------|-----------|-------------------------|
|                                   | Grams                             | Ounces | In<br>Protein            | In<br>Fat | In<br>Carbo-<br>hydrate |
| Almonds.....                      | 16                                | 0.6    | 11.6                     | 76.1      | 12.3                    |
| Apples.....                       | 156                               | 5.5    | 1.9                      | 5.6       | 92.5                    |
| Bacon.....                        | 17                                | 0.6    | 16.7                     | 82.6      | 0.7                     |
| Bananas.....                      | 101                               | 3.6    | 4.9                      | 1.8       | 93.3 <sup>a</sup>       |
| Beans, dried.....                 | 29                                | 1.0    | 25.2                     | 3.9       | 70.9                    |
| Beans, string.....                | 237                               | 8.4    | 22.7                     | 4.3       | 73.0                    |
| Beef, lean.....                   | 66                                | 2.3    | 52.3                     | 47.7      | —                       |
| Bread, white.....                 | 38                                | 1.3    | 13.0                     | 6.9       | 80.1                    |
| Broccoli.....                     | 270                               | 9.5    | 35.7                     | 4.9       | 59.4                    |
| Butter.....                       | 14                                | 0.5    | 0.3                      | 99.5      | 0.2 <sup>b</sup>        |
| Cabbage.....                      | 350                               | 12.4   | 19.6                     | 6.3       | 74.1                    |
| Carrots.....                      | 224                               | 7.9    | 10.7                     | 6.0       | 83.3                    |
| Cashew nuts.....                  | 16                                | 0.6    | 12.9                     | 69.8      | 17.3                    |
| Cheese, Cheddar,<br>American..... | 25                                | 0.9    | 24.3                     | 74.0      | 1.7                     |
| Codfish.....                      | 144                               | 5.1    | 94.8                     | 5.2       | —                       |
| Corn flakes.....                  | 28                                | 1.0    | 8.8                      | 1.8       | 89.4                    |
| Eggs.....                         | 64                                | 2.3    | 32.5                     | 65.7      | 1.8                     |
| Grapefruit.....                   | 226                               | 8.0    | 4.5                      | 4.1       | 91.4                    |
| Kale.....                         | 201                               | 7.1    | 31.3                     | 10.8      | 57.7                    |
| Lettuce.....                      | 549                               | 19.4   | 26.4                     | 9.9       | 63.7                    |
| Milk.....                         | 146                               | 5.2    | 20.4                     | 51.1      | 28.5                    |
| Oatmeal.....                      | 25                                | 0.9    | 14.3                     | 16.8      | 68.9                    |
| Oils, salad.....                  | 11                                | 0.4    | —                        | 100.      | —                       |
| Oranges.....                      | 199                               | 7.0    | 7.2                      | 3.6       | 89.2 <sup>a</sup>       |
| Oysters.....                      | 124                               | 4.4    | 48.5                     | 22.3      | 29.2                    |
| Peanuts.....                      | 17                                | 0.6    | 18.0                     | 66.3      | 15.7                    |
| Peas.....                         | 99                                | 3.5    | 26.5                     | 3.6       | 69.9                    |
| Potatoes.....                     | 117                               | 4.1    | 9.4                      | 1.0       | 89.6                    |
| Raisins.....                      | 34                                | 1.2    | 3.1                      | 1.5       | 95.4 <sup>a</sup>       |
| Salmon.....                       | 59                                | 2.1    | 48.8                     | 51.2      | —                       |
| Sugar, granulated.....            | 25                                | 0.9    | —                        | —         | 100.                    |
| Tomatoes.....                     | 441                               | 15.6   | 17.6                     | 11.9      | 70.5 <sup>a</sup>       |
| Wheat, shredded.....              | 27                                | 1.0    | 11.3                     | 3.4       | 85.3                    |

<sup>a</sup> As is usual in calculations of this kind, organic acids are here counted with carbohydrates.

<sup>b</sup> Includes both the lactose and the lactic acid of that part of the buttermilk which the butter retains.

an ounce of olive oil to over three pounds of lettuce. On the other hand, however, the lettuce and the cabbage have important mineral and vitamin values of which olive oil and clear fat pork have little if any.



### **Energy Requirement in Metabolism — Methods of Study and Amounts Required for Maintenance at Rest**

We know definitely from accurate experiments that the physiological fuel values which have been deduced represent the energy which is actually obtained by the normal body from the food and which appears as muscular work or as heat; and we have every reason to suppose that under ordinary conditions the carbohydrates, fats, and proteins each supply the body with the kinds of energy needed for its maintenance and for its work, approximately in proportion to their fuel values as calculated above. We do not now believe that any one nutrient is used to the exclusion of others as a source of energy for any particular function, nor indeed that the body makes any great distinction between the foodstuffs as sources of energy. The fuel value of the diet as a whole is utilized to meet the energy requirements of the whole body. For the present, therefore, it is the fuel value of the day's dietary which we have to consider rather than the distribution of this as regards protein, fat, and carbohydrate.

The total food (or energy) requirement is best expressed in Calories per day, either for the whole body or per kilogram of body weight, and for convenience of discussion it is usually assumed that the average body weight (without clothing) is for men 70 kilograms (154 pounds) and for women eight tenths as much, 56 kilograms (123 pounds).

There are four important methods of studying the food requirements of man:\*

1. By observing the amount of food consumed (dietary studies).
2. By observing the amount of oxygen consumed, or carbon dioxide produced, or both (respiration experiments).
3. By determining the balance of intake and output (carbon and nitrogen balance experiments).
4. By direct measurement of heat given off by the body (calorimeter experiments).

**1. Dietary studies.** Most dietary studies give little more than a general indication of the food habits of the people studied; but in cases where persons have maintained for a long time the same dietary habits and other conditions of life, and the body

\* For an account of the historical development of the principles which underlie the measurement of metabolism see the introductory chapter of Lusk's *Elements of the Science of Nutrition*.

weight has remained practically constant, it may be fairly safe to assume that the food has furnished just about the right amount of energy for the maintenance of the body under the observed conditions.

Great care must be taken in drawing inferences from the body weight because of the readiness with which the body gains or loses moisture. Athletes often lose 2 or 3 pounds in an hour of vigorous exercise and regain it in less than a day. Gain or loss of body weight during short periods, therefore, does not by any means necessarily imply a corresponding gain or loss of fat. The body may lose fat and at the same time maintain its weight through gaining water, or vice versa. When, however, the weight remains nearly the same for months at a time, it may usually be assumed that there is no important gain or loss of tissue and that the body is receiving just about the proper total amount of food for its needs. Under these conditions an accurate observation of the food consumed may give valuable indications as to the actual food requirement. Of such dietary studies perhaps the most useful individual example is that of Neumann, who reduced his diet to what appeared to be just about sufficient for his needs and then recorded all food and drink taken during a period of 10 months in which the body weight remained nearly constant. The average daily food furnished.\*

| NUTRIENTS      | WEIGHT      | FACTORS | CALORIES | TOTAL CALORIES<br>PER DAY |
|----------------|-------------|---------|----------|---------------------------|
| Protein        | 66.1 grams  | × 4. =  | 264.4    | 2242                      |
| Fat            | 83.5 grams  | × 9. =  | 751.5    |                           |
| Carbohydrate * | 306.5 grams | × 4. =  | 1226.0   |                           |

\* Including some alcohol (taken in the form of beer), which is estimated as equivalent in fuel value to 1.75 times its weight of carbohydrate.

The 2242 Calories per day were evidently fully sufficient to meet the energy requirements of this man, whose weight was 66.5 to 67 kilograms (about 147 pounds) and who was engaged at his usual (mainly sedentary) professional work in the Hygienic Institute at Kiel.

Later, when his weight had increased to 71.5 kilograms (157 pounds) as the result of following for a time a more liberal diet (furnishing about 2600 Calories per day), he again observed his dietary while taking what was supposed to be an amount of food sufficient for the maintenance of the body and no more. This

\* The data are taken from Chittenden's *Nutrition of Man*, page 286.

second dietary study was continued for 8 months, during which the average daily food consumption was found to be:

| NUTRIENTS                 | WEIGHT      | FACTORS     | CALORIES  | TOTAL CALORIES<br>PER DAY |
|---------------------------|-------------|-------------|-----------|---------------------------|
| Protein                   | 76.2 grams  | $\times 4.$ | $= 304.8$ | 2000                      |
| Fat                       | 109.0 grams | $\times 9.$ | $= 981.0$ |                           |
| Carbohydrate <sup>a</sup> | 178.6 grams | $\times 4.$ | $= 714.4$ |                           |

<sup>a</sup> Including some alcohol (taken in the form of beer), which is estimated as equivalent in fuel value to 1.75 times its weight of carbohydrate.

The body weight remained nearly constant.

These results indicate that this subject, a man of average size, living a normal professional life involving no manual labor in the ordinary sense, but not excluding such muscular movements as are naturally incidental to a sedentary occupation, found his energy requirements satisfied with food furnishing 2000 to 2250 Calories per day.

**2. Respiration experiments.** Since the foodstuffs yield their energy through being oxidized in the body, it is evident that a measure of the energy metabolism can be obtained by finding

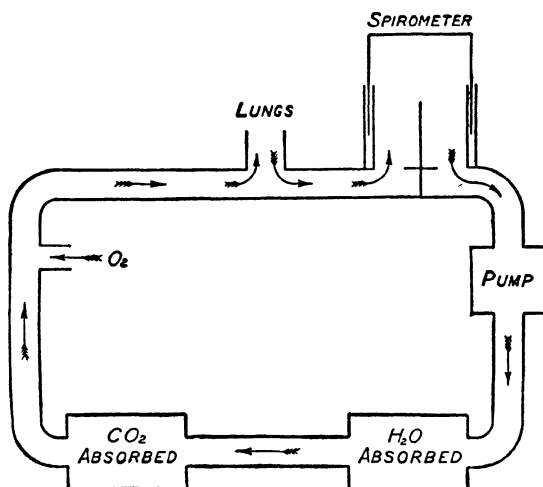


FIG. 14. — Diagram of Benedict respiration apparatus. (Courtesy of Dr. F. G. Benedict.)

either the amount of foodstuffs oxidized or the amount of oxygen which is consumed in the process. The apparatus devised and used by Zuntz for this purpose provides a mask, fitting air tight over the mouth and nose and connected by means of valved pipes

with apparatus for measuring and analyzing the inspired and expired air. In this way one can determine the volume of oxygen entering, and the volume leaving, the lungs. The difference is the volume consumed in the body.

Benedict has devised an improved form of respiration apparatus in which the subject breathes, either through a mouth- or nose-piece, from a current of air which is purified and kept in circulation in the same manner as that of the respiration calorimeter chamber described below. The carbon dioxide which the man produces is absorbed quantitatively and the oxygen which he consumes is measured by the change in level of a spirometer previously filled with oxygen gas which drops as oxygen is consumed by the man.

A given volume of oxygen used in the body may liberate somewhat different amounts of heat, according as it oxidizes fat, carbohydrate, or protein. For accurate estimations of the energy liberated it is therefore necessary to know the kind of material oxidized, as well as the amount of oxygen consumed. This is calculated from the respiratory quotient as explained in Chapter VII.

Since the amount of protein broken down in the body can be estimated from the nitrogen excretion, the determination of the respiratory quotient along with the oxygen consumption shows the extent of the combustion in the body and the proportions of fat and carbohydrate burned.\* From these data the energy can be calculated.

As a matter of fact it is not necessary to go through the actual calculation of the amounts of fat and carbohydrate burned, since the energy derived from a liter of oxygen when used to burn carbohydrate and fat in different proportions can be calculated once for all and expressed in relation to the respiratory quotient as shown in Table 11.

It is then only necessary to determine the respiratory quotient, or if working under conditions for which it is known, the volume of oxygen consumed, in order to know the number of Calories of energy metabolized. This is sometimes called the *method of indirect*

\* Or, with very little error, it may be assumed that 15 per cent of the oxygen consumed goes to burn protein and the rest is divided between fat and carbohydrate. The values given in the table herewith agree with this assumption. Attention should be called to the fact that estimates of energy metabolism based on carbon dioxide production alone involve larger errors than those based on oxygen consumption alone.

TABLE 11. — ENERGY VALUES OF OXYGEN AND CARBON DIOXIDE AT DIFFERENT RESPIRATORY QUOTIENTS (Zuntz and Schumburg)

| RESPIRATORY<br>QUOTIENT | CALORIES<br>PER LITER OF<br>OXYGEN | CALORIES<br>PER LITER OF<br>CARBON DIOXIDE | CALORIES<br>PER GRAM OF<br>CARBON DIOXIDE |
|-------------------------|------------------------------------|--|---|
| 0.70                    | 4.686                              | 6.694                                      | 3.408                                     |
| 0.71                    | 4.690                              | 6.606                                      | 3.363                                     |
| 0.72                    | 4.702                              | 6.531                                      | 3.325                                     |
| 0.73                    | 4.714                              | 6.458                                      | 3.288                                     |
| 0.74                    | 4.727                              | 6.388                                      | 3.252                                     |
| 0.75                    | 4.739                              | 6.319                                      | 3.217                                     |
| 0.76                    | 4.752                              | 6.253                                      | 3.183                                     |
| 0.77                    | 4.764                              | 6.187                                      | 3.150                                     |
| 0.78                    | 4.776                              | 6.123                                      | 3.117                                     |
| 0.79                    | 4.789                              | 6.062                                      | 3.086                                     |
| 0.80                    | 4.801                              | 6.001                                      | 3.055                                     |
| 0.81                    | 4.813                              | 5.942                                      | 3.025                                     |
| 0.82                    | 4.825                              | 5.884                                      | 2.996                                     |
| 0.83                    | 4.838                              | 5.829                                      | 2.967                                     |
| 0.84                    | 4.850                              | 5.774                                      | 2.939                                     |
| 0.85                    | 4.863                              | 5.721                                      | 2.912                                     |
| 0.86                    | 4.875                              | 5.669                                      | 2.886                                     |
| 0.87                    | 4.887                              | 5.617                                      | 2.860                                     |
| 0.88                    | 4.900                              | 5.568                                      | 2.835                                     |
| 0.89                    | 4.912                              | 5.519                                      | 2.810                                     |
| 0.90                    | 4.924                              | 5.471                                      | 2.785                                     |
| 0.91                    | 4.936                              | 5.424                                      | 2.761                                     |
| 0.92                    | 4.948                              | 5.378                                      | 2.738                                     |
| 0.93                    | 4.960                              | 5.333                                      | 2.715                                     |
| 0.94                    | 4.973                              | 5.290                                      | 2.693                                     |
| 0.95                    | 4.985                              | 5.247                                      | 2.671                                     |
| 0.96                    | 4.997                              | 5.205                                      | 2.650                                     |
| 0.97                    | 5.010                              | 5.165                                      | 2.629                                     |
| 0.98                    | 5.022                              | 5.124                                      | 2.609                                     |
| 0.99                    | 5.034                              | 5.085                                      | 2.589                                     |
| 1.00                    | 5.047                              | 5.047                                      | 2.569                                     |

*calorimetry.* One form of apparatus is shown in Fig. 15. For descriptions of other, including simpler, forms of respiration apparatus see DuBois' *Basal Metabolism in Health and Disease*.

This method of studying the energy metabolism permits of experiments being carried out very quickly, and is therefore especially useful for the direct investigation of conditions which affect energy metabolism promptly, such as muscular work or the eating of food. The periods of observation cannot be very long,

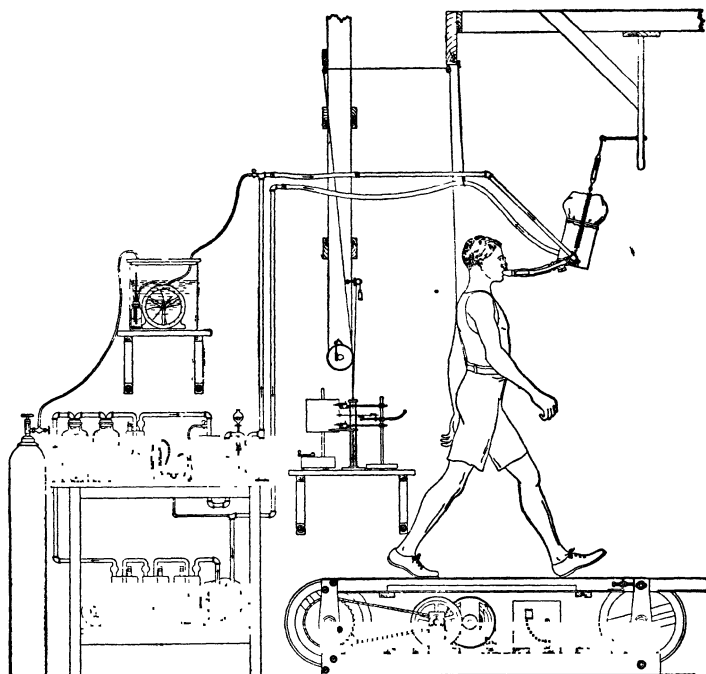


FIG. 15. — General view of apparatus used in walking experiments, with subject in position. (Courtesy of Dr. F. G. Benedict.)

but the probable results for the 24 hours' metabolism can be estimated by the data obtained during frequent short periods at different times of the day and night.

From the results of many observations by the Zuntz method Magnus-Levy estimated the minimum metabolism of a man of average size kept absolutely motionless and fasting at 1625 Calories per day. Food barely sufficient for maintenance would increase this by 175, and such incidental muscular movements as would ordinarily be made by a man at rest in bed would involve another 200, making a total of 2000 Calories as the estimated food requirement of a man at rest with a maintenance diet. Magnus-Levy further estimated that the man, if doing no work (in the ordinary sense), but allowed to move about the room instead of remaining in bed, would require 2230 Calories per day.

**3. Carbon and nitrogen balance experiments.** From a comparison of the constituents of the food consumed ("intake") and

of the substances eliminated from the body ("output"), the material actually oxidized and the energy liberated in the oxidation may be determined.

The intake is found by weighing and analyzing all food eaten; the output by collecting and determining the end products eliminated through the lungs, the kidneys, the intestines, and sometimes (in very exact experiments) the skin. The time unit in experiments upon the intake and output is almost always 24 hours, the experimental day beginning preferably just before breakfast. The feces belonging to the experimental days are marked, usually by giving a small amount of lampblack with the food as in ordinary digestion experiments, separated, and analyzed. The end products given off by the lungs and kidneys during an experimental day are taken as measuring the material broken down in the body during the same period.

Some time is of course required for the elimination of the nitrogenous end products through the kidneys. This unavoidable "lag" in the elimination of nitrogen may introduce an error in interpreting the nitrogen balance unless the subject has been kept for a few days in advance upon the same diet which is to be used in the experiment.

Assuming that the total nitrogen and carbon of the absorbed food existed in the form of protein, fat, and carbohydrate, and that the amount of carbohydrate in the body is the same at the beginning and end of each experiment, it is only necessary to determine the carbon dioxide of the expired air and the carbon and nitrogen of the waste products, in order to calculate the amounts of material oxidized and of energy liberated in the body. Experiments of this sort have played an important part in the development of our knowledge of nutrition. The calculations are usually based on the average analyses of protein and body fat shown in Table 12.

TABLE 12. — AVERAGE ELEMENTARY COMPOSITION OF PROTEIN AND FAT

|                    | PROTEIN | FAT  |
|--------------------|---------|------|
| Carbon . . . . .   | 53      | 76.5 |
| Nitrogen . . . . . | 16      | —    |
| Hydrogen . . . . . | 7       | 12   |
| Oxygen . . . . .   | 23      | 11.5 |
| Sulfur . . . . .   | 1       | —    |
|                    | 100     | 100  |

The data of Table 13 were obtained with a man on ordinary mixed diet.

A loss of 2.0 grams of body nitrogen indicates ( $2.0 \times 6.25 =$ ) 12.5 grams of body protein burned. Also there were 89.0 grams absorbed from food, and, therefore, in all 101.5 grams of total protein burned.

TABLE 13. — CALCULATION OF ENERGY METABOLISM FROM CARBON AND NITROGEN BALANCES. MAN OF 64 KILOGRAMS AT REST IN ATWATER RESPIRATION APPARATUS

| INTAKE                | GRAMS PER DAY |      |                   |          |        |
|-----------------------|---------------|------|-------------------|----------|--------|
|                       | Protein       | Fat  | Carbo-<br>hydrate | Nitrogen | Carbon |
| Total in food . . .   | 94.4          | 82.5 | 289.8             | 15.1     | 239.0  |
| Lost in digestion . . | 5.4           | 3.7  | 3.2               | 0.9      | 7.4    |
| Absorbed . . .        | 89.0          | 78.8 | 286.6             | 14.2     | 231.6  |
| OUTPUT                |               |      |                   |          |        |
| By lungs . . . . .    |               |      |                   |          | 207.3  |
| By kidneys . . . . .  |               |      |                   | 16.2     | 12.2   |
| Metabolized . . . . . |               |      |                   | 16.2     | 219.5  |
| Balance . . . . .     |               |      |                   | - 2.0    | + 12.1 |

Since the respiratory quotient showed that the body was in carbohydrate equilibrium at the beginning and end of each experimental day, *i.e.*, at seven o'clock each morning, it may be concluded that the amount of carbohydrate burned was the same as that absorbed from the food, *viz.* 286.6 grams per day.

From the carbon balance and the above data, therefore, we estimate the amount of fat burned as follows:

|  |                    |
|--|--------------------|
| 12.5 grams body protein yield ( $12.5 \times 53$ per cent =) | 6.6 grams carbon   |
| and there were in the absorbed food                          | 231.6 grams carbon |
| $\therefore$ total available was                             | 238.2 grams carbon |
| But total catabolized was only                               | 219.5 grams carbon |
| $\therefore$ the body stored in the form of fat              | 18.7 grams carbon  |

Since fat contains 76.5 per cent carbon, 1 gram carbon  $\approx$  1.307 grams fat.  $\therefore$  18.7 grams carbon = 24.4 grams fat.

The body therefore absorbed 78.8 grams fat  
 stored 24.4 grams fat  
 burned 54.4 grams fat



In all, the body burned per day

|                                    |                           |                      |
|------------------------------------|---------------------------|----------------------|
| 101.5 grams protein, yielding      | $(101.5 \times 4.35^* =)$ | 442 Calories         |
| 54.4 grams fat, yielding           | $(54.4 \times 9.45^* =)$  | 515 Calories         |
| 286.6 grams carbohydrate, yielding | $(286.6 \times 4.1^* =)$  | 1175 Calories        |
|                                    |                           | <u>2132 Calories</u> |

By means of the carbon and nitrogen balance Sonden and Tigerstedt studied the energy metabolism of eight resting men between nineteen and forty-four years of age, with results which varied for the different subjects from 1853 to 2292 Calories per day. Many other experimenters have used the same method with similar results.

**4. Calorimeter experiments.** The most direct, and in some respects most convincing, way of ascertaining the energy metabolism is by the *method of direct calorimetry*. This consists in measuring the total energy expenditure of the body as heat or as heat and mechanical work by confining the subject in a chamber permitting of actual measurement of the heat produced. It was not until the development of the Atwater-Rosa-Benedict respiration calorimeter that complete and satisfactory data covering periods of one to several days were obtained. This apparatus consisted of an air-tight copper chamber, surrounded by zinc and wooden walls with air-spaces between, and was large enough for a man to live in without discomfort, being about 7 feet long, 4 feet wide, and  $6\frac{1}{2}$  feet high. An opening in the front of the apparatus, which was sealed during an experiment, served as both door and window, admitting sufficient light for reading and writing. A smaller opening, having tightly fitting caps on both ends, was used for passing food, drink, excreta, etc., into and out of the chamber. The chamber was furnished with a folding bed, chair, and table, and was ventilated by means of a current of air which passed usually at the rate of about  $2\frac{1}{2}$  cubic feet per minute. At first this ventilating air current was maintained and measured by means of a specially constructed meter pump which also automatically took samples of the air for analysis. Later, the apparatus was so modified as to make use of the same air throughout an experiment, the carbon dioxide and water given off by the subject

\* Here the factors for fuel value are not reduced to allow for loss in digestion, because this loss has already been deducted in computing the amount of each nutrient actually absorbed and rendered available.

being removed by circulating the air through purifying vessels, and the oxygen which the subject used being replaced by adding weighed amounts of oxygen to the air current as required. By this means it is possible to carry out, in the calorimeter, metabolism experiments in which the oxygen and hydrogen as well as the carbon and nitrogen balances are determined, and from these data the gain or loss of carbohydrate as well as of protein and fat can be determined.

The ventilating air current is so regulated that it enters and leaves the calorimeter at the same temperature; and between the copper and zinc walls are placed a large number of thermo-electric junctions connected with a delicate galvanometer by means of which each wall is tested every four minutes, day and night, during the progress of an experiment, and the mere traces of heat which may pass to or from the calorimeter through its walls are quickly detected and made to balance each other. Thus there is no gain or loss of heat either through the walls of the chamber or by the ventilating air current, and the heat given off by the subject can leave only by the means especially provided for carrying it out and measuring it. A part of the heat liberated is carried from the chamber in latent form by the water vapor in the outgoing air, which is accurately determined. The rest of the heat is brought away by means of a current of cold water circulating through a copper pipe coiled near the ceiling of the chamber. The quantity of water which passes through the pipe and the difference between the temperature at which it enters and that at which it leaves the coil are carefully determined and show how much heat is thus brought out of the chamber.

In recent years several different calorimeters, based on the principles of the apparatus just described but adapted in size and shape to different types of experimentation, have come into use. Notable among these are the "chair" and the "bed" calorimeters, which are so constructed as to accommodate a subject in the sitting or reclining position in comfort but in a minimum of space; for only by making the calorimeter chamber small is it practicable to obtain a high degree of accuracy in experiments of a few hours' duration. Figures 16, 17, and 18 show a sectional diagram of the original respiration calorimeter, a photograph of the original Atwater-Rosa-Benedict apparatus, and a photograph of the more recent DuBois apparatus, respectively.

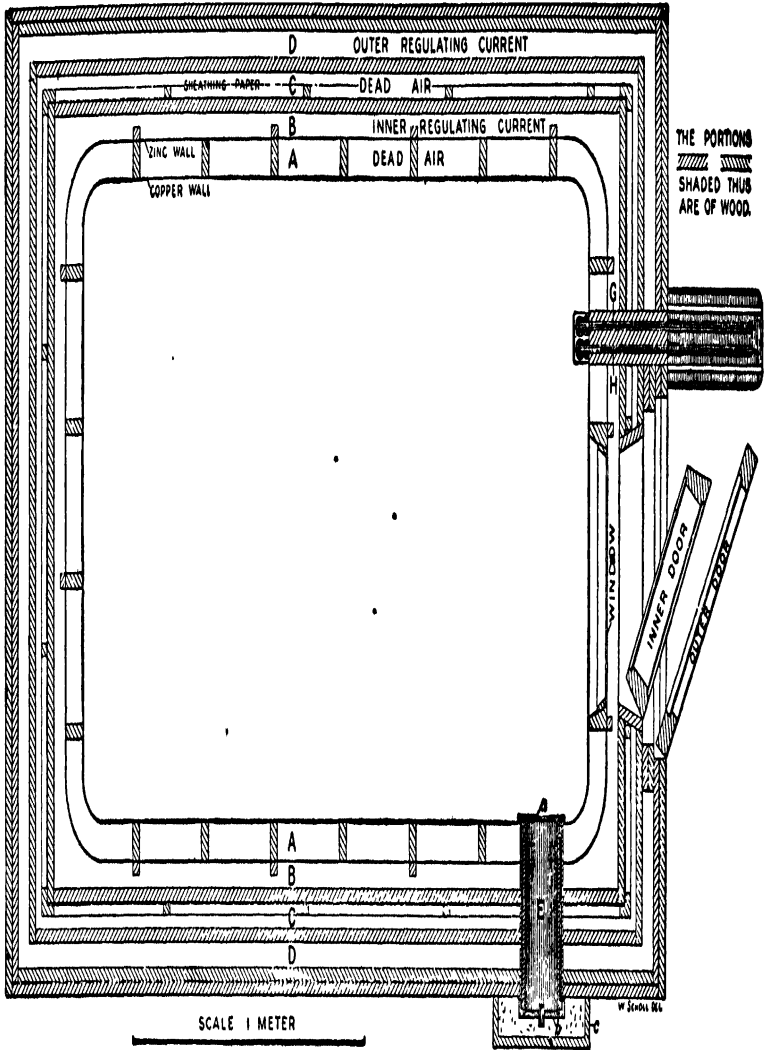


FIG. 16. — Horizontal section of the original Atwater-Rosa-Benedict respiration calorimeter. (Courtesy of the United States Department of Agriculture.)

Detailed descriptions of the chief forms of apparatus may be found in the publications listed at the end of this chapter.

By means of the Atwater-Rosa-Benedict apparatus and its various modifications, it has been possible to measure the heat production or energy expenditure of a man for a day or for a period of days very accurately. In the original Atwater-Benedict series it was found that the difference in results determined by direct and indirect calorimetry was rarely as much as 2 per cent, and in

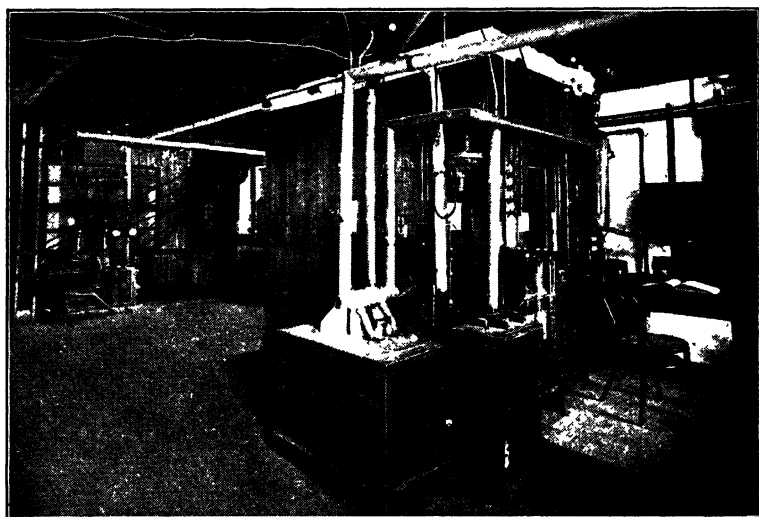


FIG. 17.—The original Atwater-Rosa-Benedict respiration calorimeter. (Courtesy of the United States Department of Agriculture.)

the average of 45 experiments covering a total of 143 days the difference was only 0.01 per cent. Hence results obtained by direct energy measurements are essentially the same as those computed from respiration and metabolism experiments when the technique is of the best and the experiments are sufficiently prolonged. This agreement is in general less exact in individual experiments in proportion as the experimental periods are shortened; but the methods are now so highly developed that the results of direct and indirect calorimetry are considered practically interchangeable even for experiments of a few hours' duration. In 1913 Armsby compiled the following summary of experiments upon men, dogs, and cattle which had been published up to that time (Table 14 on page 144). It will be seen that the difference between the total heat

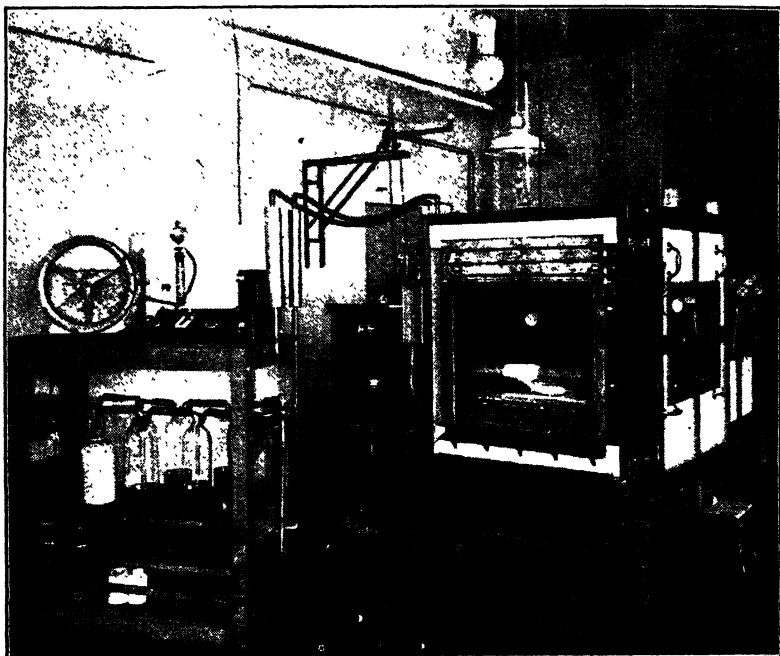
*Paul Parker Photo*

FIG. 18. — The respiration calorimeter now in use at the Russell Sage Institute of Pathology. (Courtesy of Dr. E. F. DuBois.)

production as computed and as directly measured is only one fourth of one per cent, or quite within the limits of accuracy of experimental methods of this sort.

TABLE 14. — ARMSBY'S COMPARISON OF ENERGY COMPUTED AND FOUND

| EXPERIMENTER            | TOTAL<br>NUMBER<br>OF DAYS | TOTAL<br>COMPUTED<br>HEAT<br>PRODUCTION<br>CALORIES | TOTAL<br>OBSERVED<br>HEAT<br>PRODUCTION<br>CALORIES | PERCENTAGE<br>DIFFERENCE |
|-------------------------|----------------------------|---|---|--------------------------|
| Rubner . . . . .        | 45                         | 17,406  | 17,350  | — 0.32                   |
| Laulanié . . . . .      | 7                          | 1,865   | 1,859   | — 0.31                   |
| Atwater and Benedict .  | 93                         | 249,063   | 248,930   | — 0.05                   |
| Benedict and Milner . . | 24                         | 95,075  | 95,689  | + 0.65                   |
| Benedict . . . . .      | 53                         | 102,078   | 101,336   | — 0.73                   |
| Armsby and Fries . . .  | 114                        | 976,204   | 980,234   | + 0.41                   |
|                         | 336                        | 1,441,691   | 1,445,398   | + 0.26                   |

As Armsby points out: "These results may be taken as demonstrating that the animal heat arises exclusively from the combus-

tions in the body, but they have a much broader significance. They show that the transformations of chemical energy into heat and work in the animal body take place according to the same general laws and with the same equivalencies as in our artificial motors and in lifeless matter generally. The great law of the conservation of energy rules in the animal mechanism, whether in man, carnivora, or herbivora, just as in the engine. The body

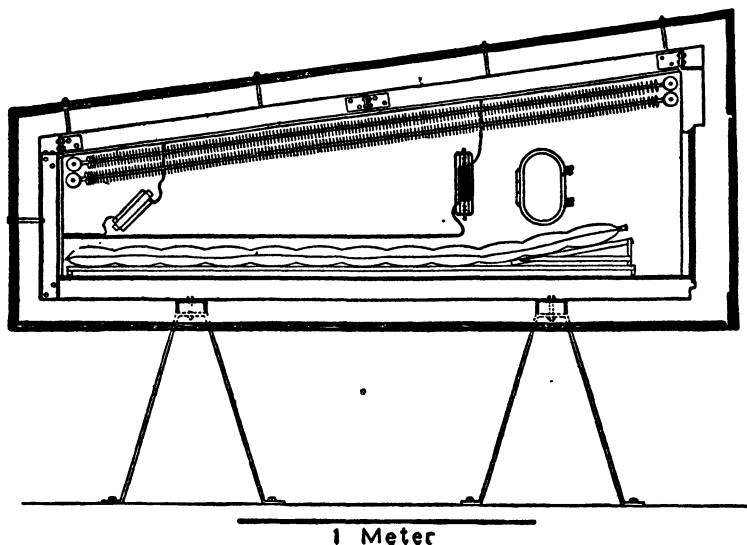


FIG. 19. — Vertical cross-section of bed calorimeter, showing part of steel construction, also copper and zinc walls, food aperture, and wall and air-resistance thermometers, and heat absorbers. (Courtesy of Dr. F. G. Benedict and the Carnegie Institution of Washington.)

neither manufactures nor destroys energy. All that it gives out it gets from its food, and all that is supplied in its food is sooner or later recovered in some form."

Since the time of Armsby's compilation, the agreement between the observed and computed heat production has been confirmed in many additional experiments, and both by the same and different experimenters.

While a large volume of later data might now be used if desired, it will be of interest to illustrate here by citing the data of pioneer experiments with men, by Atwater and Benedict. Working with the original Atwater-Rosa-Benedict calorimeter, they conducted

"rest" experiments upon six different men who lived in the calorimeter as quietly as was feasible for days at a time, taking as a rule but little more exercise than was involved in dressing and undressing, folding and unfolding the bed, table, and chair, taking samples and observations pertaining to the experiment, writing, etc., in short, the life of a healthy man, confined to one small room.

TABLE 15. — SOME RESULTS OF EXPERIMENTS BY ATWATER AND BENEDICT

| SUBJECT                                | AGE<br>YEARS | WEIGHT<br>AVERAGE    | NUMBER<br>OF EXPER-<br>IMENTS | TOTAL EX-<br>PERIMENTAL<br>DAYS | CALORIES<br>PER DAY |
|--|--------------|----------------------|-------------------------------|---------------------------------|---------------------|
| E. O. . . . .                          | 31-34        | 70 K.<br>(154 lb.)   | 13                            | 42                              | 2283                |
| A. W. S. . . . .                       | 22-25        | 70 K.<br>(154 lb.)   | 4                             | 9                               | 2337                |
| J. F. S. . . . .                       | 29           | 65 K.<br>(143 lb.)   | 4                             | 12                              | 2133                |
| J. C. W. . . . .                       | 21           | 76 K.<br>(168 lb.)   | 1                             | 4                               | * 2397              |
| H. F. . . . .                          | 54           | 70 K.<br>(154 lb.)   | 1                             | 3                               | 1904                |
| B. F. D. . . . .                       | 23           | * 67 K.<br>(147 lb.) | 1                             | 3                               | 2228                |
| Mean of individ-<br>ual averages . . . |              |                      |                               |                                 | 2213                |

The average daily metabolism of each of the subjects was as shown in Table 15.

Extreme deviations from the mean, + 184 to - 309 Calories  
or + 8.4 to - 14 per cent.

Omitting the results obtained with the one subject who was considerably older than the others, the figures become as follows:

Mean of individual averages, 2277 Calories.

Extreme deviations from mean, + 120 to - 144 Calories,  
or + 5.2 to - 6.3 per cent.

Deviations in body weight + 8.7 to - 7.1 per cent.

The subject "H. F.," aged fifty-four, who believed that he consumed only half the usual amount of food, had a food requirement about 15 per cent less than that of the younger men averaging about the same weight. The five younger men varied in age

from twenty-one to thirty-four years, were natives of three different countries, and had been accustomed to very different dietary habits and modes of life, yet they differed less in energy requirements than in body weight.

### Summary of the Evidence Obtained by the Different Methods

A general view of the results obtained by all four of the methods described shows them to be strikingly consistent and leads to the conclusion that the food requirements of a young to middle-aged man of average size, without muscular work, eating a mixed diet sufficient to meet his need, approximates 2000 Calories per day, and that such muscular activity as is incidental to very quiet living indoors may be expected to raise this requirement to about 2200 Calories per day.

Lusk summarized the mean energy requirement of an average-sized man in somewhat more precise terms as follows:

|  |               |
|--|---------------|
| Absolute rest in bed without food                            | 1680 Calories |
| Absolute rest in bed with food                               | 1840 Calories |
| Rest in bed 8 hours, sitting in chair 16 hours,<br>with food | 2168 Calories |

The very close agreement in results reached by many independent investigators, using four distinct methods of study, had thus established the approximate average food requirement of a man at rest beyond any reasonable doubt, by the end of the first decade of this century. Since that time, and especially since the general introduction of relatively simple and inexpensive forms of respiration apparatus there has been much development of our knowledge of the causes and extent of individual differences and the environmental conditions which influence energy metabolism. These topics will be considered in the next chapter.

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## CHAPTER IX

### THE BASAL ENERGY METABOLISM, REGULATION OF BODY TEMPERATURE, AND SPECIFIC DYNAMIC ACTION

THE TERM *basal metabolism* is used to designate the energy metabolism of the body when at complete rest (both mentally and physically) in the so-called "post-absorptive state" (12 to 18 hours after the last intake of food) in a room of comfortable temperature and when the body temperature is within normal range. If this last condition is not satisfied, the deviation from the normal temperature must be determined. This basal energy metabolism for a given age and size is used as the starting point for the calculation of the total energy requirement of individuals of different muscular activities and also as a basis of comparison in pathological disturbances.

Such analyses as have been made of the maintenance requirement of the body, with reference to its principal functions, indicate that in the healthy adult the basal expenditure of energy may be attributed in part, perhaps about one third, to the functional activities of the various organs (heart action, kidney action, respiration, etc.). The greater part of the basal heat production is thought to be due to oxidations in the resting tissues, principally in maintaining the tone of the skeletal muscles. In the healthy adult the basal metabolism depends chiefly upon the intensity of these internal processes and the size, shape, and composition of the body.

**Influence of internal activities.** The work of maintaining the respiration and circulation obviously involves a continual expenditure of energy. It is clear too that deep and rapid breathing or vigorous heart action must involve an increased activity of the muscles concerned. But it is not always clear to what extent increased respiratory and heart action are a cause and to what extent they are an effect of increased energy metabolism. Thus Murlin

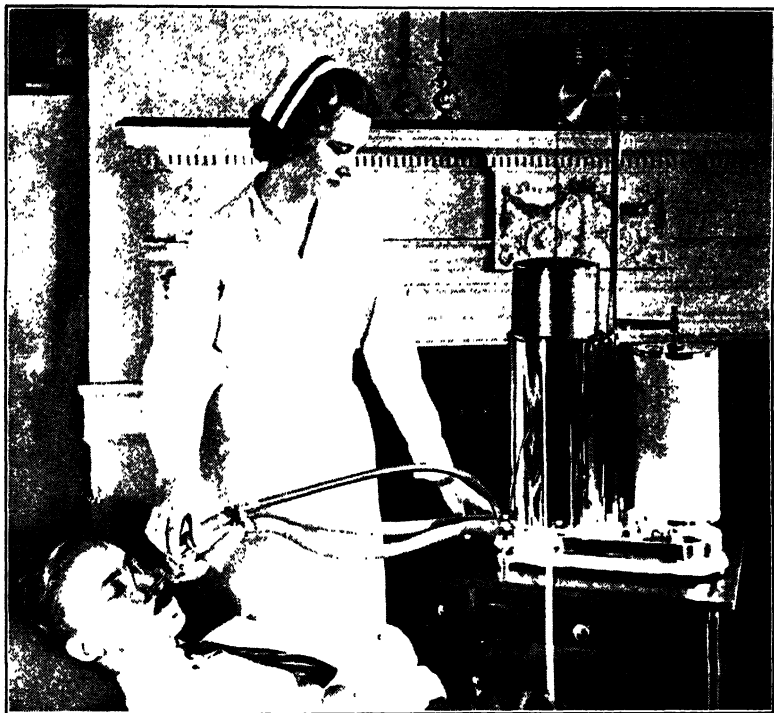


FIG. 20. — Benedict-Roth portable respiration apparatus for determination of basal metabolism. (Courtesy of Warren E. Collins, Inc.)

and Greer \* emphasize the close relationship of the heart to the requirements of the tissues for energy in that the energy metabolism is immediately dependent upon oxygen supply. Since but little available oxygen can be stored in the living substance, "the response of the heart to variations in the (energy) requirement must be immediate and, within very narrow limits of time, proportional to this requirement."

A large factor in basal metabolism is the maintenance of muscular tension or tone. That every living muscle is always in a state of tension is evident from the fact that it gapes open if cut. It is equally evident that the degree of tension (and therefore the expenditure of energy required to maintain it) varies greatly in different individuals under similar conditions and in the same individual under different conditions. The differences observed by Atwater

\* *American Journal of Physiology*, Vol. 33, page 253.

and Benedict between the metabolism of the sleeping hours and that of the hours spent sitting up without muscular movement (65 and 100 Calories respectively) are largely due to the more complete relaxation of the muscles during sleep. Thus there is in the "resting" muscle a continual expenditure of energy which first takes the form of muscular tension, or tone, but ultimately appears as heat, so that the heat production, or energy metabolism, of the body at rest depends to a considerable extent upon the degree of tension which still persists in the muscles.

Benedict and Carpenter report the data cited in Table 16 for the energy metabolism during sleep (1 A.M. to 7 A.M.) following different conditions of activity and showing the after effects of work upon muscular tension during rest.

TABLE 16. — ENERGY METABOLISM DURING SLEEP — CALORIES PER HOUR

| SUBJECT          | SLEEP AFTER<br>REST | SLEEP AFTER<br>MODERATE<br>WORK | SLEEP AFTER<br>SEVERE WORK | SLEEP AFTER<br>VERY SEVERE<br>WORK |
|------------------|---------------------|---------------------------------|----------------------------|------------------------------------|
| E. O. . . . .    | 69.3                | 74.8                            | —                          | —                                  |
| J. F. S. . . . . | 60.4                | 65.3                            | —                          | —                                  |
| J. C. W. . . . . | 77.2                | —                               | 83.1                       | —                                  |
| B. F. D. . . . . | 69.8                | —                               | 83.3                       | —                                  |
| A. L. L. . . . . | 78.3                | —                               | 83.7                       | 97.9                               |

**Influence of the size, shape, and composition of the body.** For different adults of the same species the basal metabolism (and therefore the total food requirement) as a rule increases with the size, but not to the same extent that the body weight increases; so that the requirement, though greater in absolute amount, is less per unit of body weight in the larger individual than in the smaller. *The basal metabolism increases in proportion to the surface rather than the weight.* Thus Rubner collected the data shown in Table 17 from experiments upon seven different dogs, all full grown but differing greatly in size.

Here the heat production in Calories per kilogram was over twice as great in the smallest as in the largest dog, but the total metabolism was nearly proportional to the surface area throughout.

That the relationship of basal metabolism to body surface is not due simply to loss of heat through the cooling effect of the environment will be apparent from the observations upon the regulation of body temperature.

TABLE 17. — RUBNER'S DATA ON ADULT DOGS OF DIFFERENT SIZES

| No. | BODY WEIGHT<br>KILOGRAMS | HEAT PRODUCTION IN CALORIES PER DAY |                                |                                     |
|-----|--------------------------|-------------------------------------|--------------------------------|-------------------------------------|
|     |                          | Total                               | Per Kilogram of<br>Body Weight | Per Square Meter<br>of Body Surface |
| I   | 3.10                     | 273.6                               | 88.25                          | 1214                                |
| II  | 6.44                     | 417.3                               | 64.79                          | 1120                                |
| III | 9.51                     | 619.7                               | 65.16                          | 1183                                |
| IV  | 17.70                    | 817.7                               | 46.20                          | 1097                                |
| V   | 19.20                    | 880.7                               | 45.87                          | 1207                                |
| VI  | 23.71                    | 970.0                               | 40.91                          | 1112                                |
| VII | 30.66                    | 1124.0                              | 36.66                          | 1046                                |

Armsby, in his *Principles of Animal Nutrition*, cites the explanation offered by von Hösslin — that the internal work and the consequent heat production in the body are substantially proportional to the two-thirds power of its volume, and since the external surface bears the same ratio to the volume, a proportionality necessarily exists between heat production and surface.

Largely as the result of Rubner's work it became customary to express basal energy requirements in terms of surface rather than of weight; but on account of the difficulties involved in actual measurements of the surface it has usually been computed from the weight. The earlier computations were made by Meeh's formula,  $S = W^{\frac{2}{3}} \times C$ , or  $S = 12.3 \sqrt[3]{W^2}$ , in which  $S$  represents surface,  $W$  the weight, and the constant 12.3 represents the average value found by Meeh in a series of measurements of men.

Lissauer's modification of Meeh's formula, based on measurements of infants, is used extensively for estimating the surface area of infants and young children:

$$S = 10.3 \sqrt[3]{W^2}.$$

Benedict and Talbot have modified the constant of this formula to adapt it to increases in body weight up to 40 kilograms.

A series of measurements of body surface made by DuBois and DuBois led them to the conclusion that Meeh's formula yields results higher than the true average. They used two methods for computing the surface: (1) from a series of nineteen measurements of different parts of the body, the surface of each part being computed and the results added together ("linear formula"), and (2) a "height-weight formula" which they derived mathematically

from the data of all available measurements of height, weight, and surface.

The height-weight formula may be written thus:

$$A = W^{0.425} \times H^{0.725} \times C$$

or in the form:

$$\text{Log } A = (\text{Log } W \times 0.425) + (\text{Log } H \times 0.725) + 1.8564$$

in either of which

$A$  = Surface area in square centimeters

$H$  = Height in centimeters

$W$  = Weight in kilograms

$C$  = A constant (71.84).

Using this formula the authors have constructed a chart (Fig. 21) from which the approximate surface area in square meters may be obtained at a glance if height and weight are known. The data given in Table 18 have been taken from this chart.

TABLE 18. — SURFACE AREA IN SQUARE METERS FOR DIFFERENT HEIGHTS AND WEIGHTS

| HEIGHT IN<br>CENTI-<br>METERS | WEIGHT IN KILOGRAMS |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|-------------------------------|---------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|                               | 25                  | 30   | 35   | 40   | 45   | 50   | 55   | 60   | 65   | 70   | 75   | 80   | 85   | 90   | 105  |
| 200                           |                     |      |      |      |      |      | 1.84 | 1.91 | 1.97 | 2.03 | 2.09 | 2.15 | 2.21 | 2.26 | 2.31 |
| 195                           |                     |      |      |      |      | 1.73 | 1.80 | 1.87 | 1.93 | 1.99 | 2.05 | 2.11 | 2.17 | 2.22 | 2.27 |
| 190                           |                     |      |      |      | 1.56 | 1.63 | 1.70 | 1.77 | 1.84 | 1.90 | 1.96 | 2.02 | 2.08 | 2.13 | 2.18 |
| 185                           |                     |      |      | 1.53 | 1.60 | 1.67 | 1.74 | 1.80 | 1.86 | 1.92 | 1.98 | 2.04 | 2.09 | 2.14 | 2.19 |
| 180                           |                     |      |      | 1.49 | 1.57 | 1.64 | 1.71 | 1.77 | 1.83 | 1.89 | 1.95 | 2.00 | 2.05 | 2.10 | 2.15 |
| 175                           | 1.19                | 1.28 | 1.36 | 1.46 | 1.53 | 1.60 | 1.67 | 1.73 | 1.79 | 1.85 | 1.91 | 1.96 | 2.01 | 2.06 | 2.11 |
| 170                           | 1.17                | 1.26 | 1.34 | 1.43 | 1.50 | 1.57 | 1.63 | 1.69 | 1.75 | 1.81 | 1.86 | 1.91 | 1.96 | 2.01 | 2.06 |
| 165                           | 1.14                | 1.23 | 1.31 | 1.40 | 1.47 | 1.54 | 1.60 | 1.66 | 1.72 | 1.78 | 1.83 | 1.88 | 1.93 | 1.98 | 2.03 |
| 160                           | 1.12                | 1.21 | 1.29 | 1.37 | 1.44 | 1.50 | 1.56 | 1.62 | 1.68 | 1.73 | 1.78 | 1.83 | 1.88 | 1.93 | 1.98 |
| 155                           | 1.09                | 1.18 | 1.26 | 1.33 | 1.40 | 1.46 | 1.52 | 1.58 | 1.64 | 1.69 | 1.74 | 1.79 | 1.84 | 1.89 |      |
| 150                           | 1.06                | 1.15 | 1.23 | 1.30 | 1.36 | 1.42 | 1.48 | 1.54 | 1.60 | 1.65 | 1.70 | 1.75 | 1.80 |      |      |
| 145                           | 1.03                | 1.12 | 1.20 | 1.27 | 1.33 | 1.39 | 1.45 | 1.51 | 1.56 | 1.61 | 1.66 | 1.71 |      |      |      |
| 140                           | 1.00                | 1.09 | 1.17 | 1.24 | 1.30 | 1.36 | 1.42 | 1.47 | 1.52 | 1.57 |      |      |      |      |      |
| 135                           | 0.97                | 1.06 | 1.14 | 1.20 | 1.26 | 1.32 | 1.38 | 1.43 | 1.48 |      |      |      |      |      |      |
| 130                           | 0.95                | 1.04 | 1.11 | 1.17 | 1.23 | 1.29 | 1.35 | 1.40 |      |      |      |      |      |      |      |
| 125                           | 0.93                | 1.01 | 1.08 | 1.14 | 1.20 | 1.26 | 1.31 | 1.36 |      |      |      |      |      |      |      |
| 120                           | 0.91                | 0.98 | 1.04 | 1.10 | 1.16 | 1.22 | 1.27 |      |      |      |      |      |      |      |      |

Nomograms for estimating the surface area from height and weight, devised by Boothby and Sandiford and by Hannon, will be found on pages 133 and 135 in the third (1936) edition of DuBois' *Basal Metabolism in Health and Disease*.

On applying the height-weight formula to the recorded energy metabolism of the large number of men studied in Benedict's

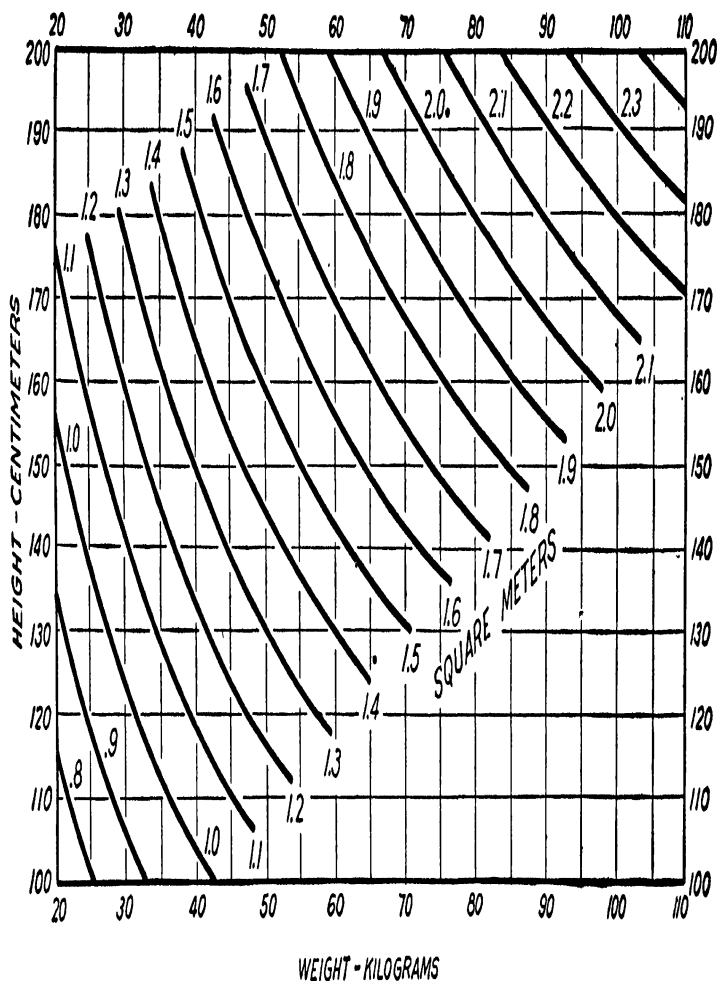


FIG. 21.—Chart for determining surface area of man from weight in kilograms and height in centimeters according to DuBois formula. (Courtesy of Dr. E. F. DuBois.)



laboratory, as well as in his own, DuBois finds that all the data for men under 50 years of age are within 15 per cent of the average basal heat production of 39.7 Calories per hour per square meter of surface area properly computed, and that 86 per cent of all the cases are within 10 per cent of the average. Means, using the more accurate linear formula, finds all of his 16 normal cases (9 men and 7 women) and also most of his obesity cases to fall within DuBois' "normal limits," *i.e.*, within 10 per cent of DuBois' average of 39.7 Calories per hour per square meter. DuBois\* believes that one may "feel certain that with men between the ages of 20 and 50 the (basal) metabolism of each individual is proportional to his surface area whether he be short or tall, fat or thin."

Bradfield has reported that the surface area of 47 young women measured by a surface integrator averaged 1.8 per cent less than that given by the DuBois height-weight formula and suggests that a 2 per cent correction should be made when it is used.

Scammon, Boyd, Klein, and Lawrence have reported measurements of the surface area of fetuses and small children from which they have developed the following formulas for computing surface area:

For fetuses:  $S = W^{0.750} \times 5.188$

For children:  $S = W^{0.575} \times H^{0.275} \times 394.56$

Brody, Comfort, and Matthews, after a detailed study of all surface area formulas, conclude that for individuals of "normal" build the best formula is

$$A = 240 \times H^{0.40} \times W^{0.53}.$$

Boyd has (1935) summarized the work on the determination of surface area in *The Growth of the Surface Area of the Human Body* to which the reader is referred for fuller treatment of the subject. Benedict (1938) in his monograph on *Vital Energetics* devotes a section to a critical examination of the relation between surface area and basal metabolism in man and other animals.

Differences of build (shape of body) are associated not only with varying ratios of weight to surface but also with differences of fatness, *i.e.*, of body composition. The thin man, besides having a

\* *American Journal of the Medical Sciences*, June, 1916, page 786.

greater surface in proportion to his weight, differs also from the stout man in that a larger percentage of his body is actual protoplasm. Since the metabolism of the body depends more upon its weight of protoplasm (active tissue) than upon its total weight, we have here an important reason for believing that the food requirement will be greater in a tall, thin man than in a shorter and fatter man of the same weight.

Von Noorden tested this question by observing the metabolism (for one day without food) of two men of different build but nearly the same weight. The results were as follows: 1st man, thin and muscular, weight 71.1 kilograms — 2392 Calories = 33.6 Calories per kilogram; 2nd man, stout, weight 73.6 kilograms — 2136 Calories = 29.0 Calories per kilogram. These two men had nearly the same weight but differed in height, in body composition, and in energy expenditure.

Even with the same height and weight there may be differences in the composition of the body. Thus a man of average height and weight but large-boned and loosely built will be of less than average fatness; a man of the same height but less broad-shouldered must be somewhat fatter in order to weigh the same. Hence equality of height and weight does not necessarily imply the same shape and composition of body. Benedict finds among normal adults of like height and weight the basal metabolism of athletes about 5 per cent higher, and that of women about 5 per cent lower, than that of average non-athletic men. He attributes these divergencies to differences in body composition, holding that women have somewhat more fat, and athletes somewhat less, than non-athletic men of the same weight and height.

**Standards for normal basal metabolism.** On the basis of the data from many determinations, tables or formulas for predicting the basal metabolism of normal individuals have been evolved by Aub and DuBois, by Harris and Benedict, by Dreyer, and by Boothby, Berkson, and Dunn.

The figures of Aub and DuBois are most significant for the age range where the greatest number of studies have been made (males from 20 to 40 years). The figures for females are not the averages of direct determinations but were calculated from an examination by Gephart and DuBois of the results of studies of the basal metabolism of women by Benedict and Emmes and by Means as 7 per cent below the average for males.

TABLE 19. — "SAGE NORMAL STANDARDS" OF AUB AND DuBOIS

| AGE IN YEARS | CAL. PER SQ. M. OF BODY SURFACE PER HOUR |         |
|--------------|--|---------|
|              | Males                                    | Females |
| 14-16        | 46.0                                     | 43.0    |
| 16-18        | 43.0                                     | 40.0    |
| 18-20        | 41.0                                     | 38.0    |
| 20-30        | 39.5                                     | 37.0    |
| 30-40        | 39.5                                     | 36.5    |
| 40-50        | 38.5                                     | 36.0    |
| 50-60        | 37.5                                     | 35.0    |
| 60-70        | 36.5                                     | 34.0    |
| 70-80        | 35.5                                     | 33.0    |

Krogh states that these Aub-DuBois standards are too high and has published a table of modified Aub-DuBois standards for the ages of 15 to 75 years with a uniform reduction of 6 per cent.\*

Harris and Benedict made a biometric study of their findings on 136 men, 103 women, and 94 infants in an attempt to evolve an accurate method of calculating unknown basal metabolism. The prediction formulas they derived are as follows:

$$\text{For males: } H = 66.473 + 13.752 W + 5.003 S - 6.755 A$$

$$\text{For women: } H = 655.096 + 9.563 W + 1.850 S - 4.676 A$$

$H$  = Total heat production in 24 hours

$W$  = Weight in kilograms

$S$  = Stature in centimeters

$A$  = Age in years

(The fact that each of these formulas is given in the literal terms of its derivation may make the comparison of them somewhat disconcerting at first glance. On working through the two formulas, however, it will be seen that there is neither a mistake of decimal point nor a great difference between the basal metabolisms of the sexes.)

Calculation of results by these formulas is much simplified by the use of tables compiled by Carpenter.†

\* Krogh's *Recording Respiration Apparatus — Tables of Normal Metabolic Rates*. J. H. Schultz, Copenhagen, 1925.

† Carpenter, *Tables, Factors and Formulas for Computing Respiratory Exchange and Biological Transformations of Energy*. Carnegie Institution of Washington, Publication No. 303 A (1924).

Krogh holds that the Harris and Benedict formulas predict accurately only for individuals within the range of weight, height, and age from which these measurements were taken. He believes that they do not hold good for individuals whose weight varies widely from the average for their height and age.

Dreyer in 1920 made a statistical study of the data of Harris and Benedict, with the object of evolving formulas which would predict basal metabolism more accurately than those of Harris and Benedict or the standards of Aub and DuBois. These formulas, which are applicable to males and females of the age of 5 years upward, are as follows:

$$\text{For males: } C = \frac{\sqrt{W}}{0.1015 \times A^{0.1333}}$$

$$\text{For females: } C = \frac{\sqrt{W}}{0.1127 \times A^{0.1333}}$$

$C$  = Calories per 24 hours

$W$  = Weight in grams

$A$  = Age in years

The average basal metabolism of 17 normal college women studied by Blunt and Dye was 6.5 per cent below the Aub-DuBois and 4.1 per cent below the Harris-Benedict standards. MacLeod and Rose found the average deviation of 92 normal women between the ages of 20 and 50 to be 8.6, 4.5, and 3.4 per cent below the Aub-DuBois, Harris-Benedict, and Dreyer predictions respectively. Boothby and Sandiford found the Aub-DuBois standards 1 to 4 per cent high for adults and 3 to 7 per cent high for children and on the basis of these findings have published a table of modified Aub-DuBois standards. Boothby, Berkson, and Dunn (1936) after statistical analysis of still more data proposed another set of standards in Calories per square meter per hour to be known as the Mayo Foundation Standards. Benedict in a study of 27 normal men and 33 normal women found that the basal metabolism of the men averaged 4.4 per cent below the Aub-DuBois and 1.8 per cent below the Harris-Benedict predictions, while that of the women averaged 7.3 and 4.2 per cent below the same standards respectively.

McKay found the basal metabolism of a group of women 35 to 50 years of age to average about 7.3 per cent below the Aub-DuBois standards for this age range, while that of a group 50 to

60 years of age showed an average deviation of -- 11.4 per cent. Jenkins has reported that analysis of the results on 2994 women and 1126 men gave average deviations of - 7.5 per cent, - 6.6 per cent, and - 6.8 per cent from the Aub-DuBois, Harris-Benedict, and Dreyer predictions respectively. Stark, in studies of the basal metabolism of a large group of young men and women 17 to 21 years of age, reaches the conclusion that the best standards for this age range are those obtained from a straight-line extrapolation of the Harris-Benedict adult standards. These findings seem quite consistent in indicating that for women the Aub-DuBois standards may be 7 to 8 per cent too high and those of Harris and Benedict, 4 to 5 per cent too high, while for men both standards may be about 4.5 per cent too high.

**Standards for children.** Standards for basal metabolism in children are far from satisfactory (1941). The Aub-DuBois standards do not extend below the age of 14 years and for girls of the ages to which they apply seem to be too high. Harris and Benedict give no figures for ages below 21 years in the prediction tables based on their formulas but experience with these formulas indicates that although they do not predict accurately for girls they seem to apply well to boys. The Dreyer formulas are applicable to both boys and girls 5 years of age and above and in general give results agreeing well with those actually obtained on normal children. For boys weighing 3 to 38 kilograms Benedict and Talbot have established standards based on body weight. For girls from 1 week to 12 years of age Benedict has proposed predicting the basal metabolism from height. A frequent difficulty in using these standards is that many 11- and 12-year-old girls are taller than the maximum height given in the table, while for the younger girls whose heights occur in the table the predictions seem to be somewhat low. On the basis of his work with groups of Girl Scouts in his respiration chamber Benedict has set up standards for girls 12 to 20 years of age predicting the basal heat production for each half-year of age, but these figures having been obtained during sleep are found to be too low when compared with actual basal metabolisms obtained under the usual standard conditions.

Talbot (F. B.) has proposed standards for boys and girls on the basis of body weight, giving the basal metabolism in Calories per 24 hours while Talbot (N. B.), Worcester, and Stewart have proposed predicting the basal metabolism of children from creatinine output.

In studies of the basal metabolism of 3- and 4-year-old children (17 girls and 12 boys) Robb found that the girls averaged 25 per cent above the Benedict standard based on height, and 9 per cent below the Dreyer standard, while the average deviation of the boys from the Benedict and Talbot standard (based on weight) was + 19 per cent, from the Dreyer standard - 13 per cent, and from the Harris-Benedict standard + 17 per cent. Williams, studying a smaller group of children of this age range, found similar deviations: for girls + 26 per cent from the Benedict (height) standard and - 10 per cent from the Dreyer standard; while for the boys the deviations were + 20 per cent from the Benedict and Talbot standard based on weight, - 13 per cent from the Dreyer, and + 19 per cent from the Harris-Benedict standard. Working with 9-year-old girls Potgieter found average deviations of + 9.1 per cent and - 5.8 per cent from the Benedict height and the Dreyer predictions respectively, while in a group of girls 10 to 12 years of age Thompson found average deviations of - 1.6 and - 8.5 per cent from these same standards. Taylor, working with 9- to 11-year-old boys, reported average deviations of - 10.1, + 4.3, and + 4.6 per cent from the Dreyer, the Benedict and Talbot, and the Harris and Benedict standards respectively. For infants Levine and Marples have found the Benedict and Talbot standards satisfactory. The best procedure at the present time seems to be that of comparing the actual basal metabolism of a child with all the standards which can be applied before saying whether or not it is within normal range.

Investigators have found variations of 10 to 15 per cent from the average basal metabolism in normal individuals of the same age group. Differences of similar magnitude are found in determinations made on the same individual over a prolonged period.

**Influence of brain and nerves.** In any consideration of this question it is important to distinguish sharply between the nervous control of muscular conditions and the metabolism of the brain and nerve substance itself. As emphasized particularly by Mathews, the brain receives a copious blood supply, and the blood coming to the brain is arterial, while that leaving the brain is venous, indicating that considerable oxidative metabolism occurs in brain tissue. Tashiro and others have shown that the carbon dioxide production of nerve fiber is increased when the nerve is stimulated to activity. But since the entire weight of brain and nerve substance constitutes

only about 2 per cent of the body weight, it remains questionable whether, even if its metabolism increases with "mental activity," the increase would be appreciable in measurements of the energy expenditure of the body as a whole. Among the best-controlled experiments upon this problem of energy expenditure are those of Benedict and Carpenter, in which 22 college students were given their mid-year examinations in the respiration calorimeter and their energy metabolism during the three-hour period covered by the examination was compared with that during the same period on another day when the student sat in the calorimeter at rest. In some individuals the metabolism was higher during the examination period, while in others it was lower — results much more likely due to involuntary increase or decrease of muscular tension than to altered metabolism of the brain tissue. In the average of the entire series of experiments there appeared a slight increase of oxygen consumption, carbon dioxide output, and heat production during the examination, but the increase was so small and the exceptions so numerous that the investigators were not willing to conclude from their results that mental work has any positive effect upon the total metabolism, but rather infer the opposite. More recent experiments by Benedict and Benedict with apparatus permitting shorter periods and more exact measurements have shown that intense mental effort increases energy expenditure about 4 per cent above the basal rate, an amount quite insignificant as compared with the increases due to ordinary muscular activities.

Apparently we must conclude that such changes in energy metabolism as may result from differences in activity of the brain and nerves involved in the performance of mental work are so small, in comparison with the energy exchanges always going on in the muscles, that the former are quite obscured by the unavoidable fluctuations of the latter, and so play no measurable part in determining the total food requirement of the body. This conclusion, however, does not exclude the probability of stimulation of the basal rate by strong emotions, acting directly to increase muscle tone or through the mechanism of the internal secretions in ways such as those suggested in the following paragraph.

**Influence of internal secretions and pathological conditions.** Some internal secretions influence the basal metabolism, notably that of the thyroid gland, thyroxine, which is quite specifically a regulator of the rate of oxidation in the body. If for any reason the

thyroid gland becomes overactive, the basal metabolism increases. This increase has been known to amount to as much as 75 per cent. If, on the other hand, the activity of the thyroid is reduced, a lowering of the basal metabolism, which may amount to as much as 30 per cent, results. Another internal secretion which affects the basal metabolism is adrenaline, but its effect is much less and of much shorter duration than that of thyroxine. Overactivity of the pituitary gland tends to result in a rise of the basal metabolism, while subnormal activity may cause a fall in the basal rate. These changes are not usually of very great magnitude and are not nearly

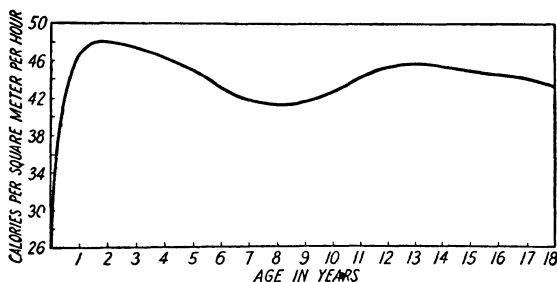


FIG. 22. — Basal metabolism of boys in Calories per square meter per hour. (Courtesy of Drs. M. S. Rose and Grace MacLeod.)

as characteristic as those due to abnormal functioning of the thyroid gland and the adrenals.

Certain pathological conditions are accompanied by a characteristic alteration in the basal rate. DuBois reports an average increase in fevers of 13 per cent for each degree Centigrade (or 7.2 per cent for each degree Fahrenheit) rise in temperature above the normal level. Basal metabolism is accelerated in exophthalmic goiter, leukemia, malarial and typhoid fevers, and in tuberculosis accompanied by fever; it is neither increased nor decreased materially in adolescent goiter, tuberculosis unaccompanied by fever, uncomplicated cases of obesity, arthritis, gout, nephritis, and cardiac and renal disorders. Hypothyroidism and myxedema are marked by a lowered heat output.\*

**Influence of age and growth.** From the fact that in animals of the same species, but of different size, the heat production is proportional to the surface rather than to the weight it would follow

\* For a more extended discussion of the clinical significance of basal metabolism determinations, see DuBois, *Basal Metabolism in Health and Disease*.



that children must have a greater maintenance requirement per unit of weight than adults.

From the data obtained in extensive studies of the basal metabolism of children, Benedict and Talbot have made charts showing the results in individual cases with curves indicating the averages.

The new-born infant has an average basal metabolic rate of 48 Calories per kilogram per 24 hours, much lower than the rate for older children. Krogh attributes the regular increase in basal

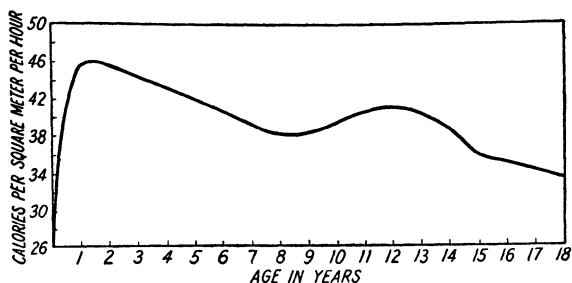


FIG. 23. — Basal metabolism of girls in Calories per square meter per hour. (Courtesy of Drs. M. S. Rose and Grace MacLeod.)

metabolism in infancy to “the development of the muscular system as such and perhaps simply the gradual development of muscle tone.” Since the rate is comparatively low during the first few months when growth is most rapid, the high metabolism of children does not seem to be due to a specific stimulus associated with growth.

There is some question whether the downward trend of the basal metabolic curve after the second year is temporarily broken during the prepubescent period. Comparative studies of boys made by DuBois before and after the onset of puberty indicate that there is a temporary rise in the prepubescent period. Benedict and Hendry, in group determinations on 105 girls, found no proof of the influence of prepubescence on the metabolic rate. MacLeod in 362 experiments upon 43 girls between eleven and fourteen years of age obtained results which indicate that girls of twelve and thirteen show an increased rate of metabolism corresponding to that shown by the boys of the DuBois studies. Göttche, Lax and Petényi, Kestner, Topper and Mulier, and Rosenblüth have reported evidence of an increase in basal metabolism at the onset of puberty. Boothby, Sandiford, and Harrington and also Blunt, Tilt, Mc-

Laughlin, and Gunn have reported finding no effect of puberty on the basal metabolism.

Table 20, compiled by Professor M. S. Rose from all available data, serves well to show the trend of changes in the basal metabolism of children with age, expressed in Calories per square meter per hour. (See also Figs. 22 and 23.)

TABLE 20. — BASAL METABOLISM OF CHILDREN  
(Data compiled by M. S. Rose)

| AGE               | CALORIES PER SQUARE METER PER HOUR |              |
|-------------------|------------------------------------|--------------|
| Premature infants | 25                                 |              |
| Birth to 2 weeks  | 25.5-29.2                          |              |
|                   | <i>boys</i>                        | <i>girls</i> |
| 3 months          | 38.7                               | 36.2         |
| 6 months          | 43.3                               | 41.2         |
| 9 months          | 46.6                               | 45.8         |
| 12 months         | 47.5                               | 46.2         |
| 15 months         | 48.1                               | 46.4         |
| 18 months         | 48.3                               | 45.8         |
| 21 months         | 48.0                               | 45.7         |
| 2 years           | 47.9                               | 45.4         |
| 3 years           | 47.1                               | 43.3         |
| 4 years           | 45.8                               | 42.5         |
| 5 years           | 44.5                               | 41.6         |
| 6 years           | 43.7                               | 41.2         |
| 7 years           | 42.9                               | 40.4         |
| 8 years           | 42.1                               | 40.0         |
| 9 years           | 41.6                               | 39.5         |
| 10 years          | 40.8                               | 37.1         |
| 11 years          | 38.9                               | 37.5-41.4    |
| 12 years          | 38.5-51.5                          | 38.2-42.4    |
| 13 years          | 38.5-46.5                          | 37.4-41.0    |
| 14 years          | 37.3-44.3                          | 36.6-40.8    |
| 15 years          | 45.3                               | 31.0-34.6    |
| 16 years          | 44.7                               | 31.0-32.3    |
| 17 years          | 43.7                               | 32.3         |
| 18 years          | 42.9                               | 32.2         |

The metabolic rate decreases throughout adult life although there is apparently a smaller drop between 20 and 30 years of age than in any other decade. The data are still insufficient to permit a definite statement as to the rate of decrease. Benedict

found the basal metabolism of a woman studied between the ages of 24 and 36 years remarkably constant, while two men studied between the ages of 30 and 57 years showed a distinct decrease as age increased, the most marked change appearing at the age of 47 in the one, of 42 in the other. In a third man, studied between the ages of 43 and 59, the metabolism remained essentially constant, due, perhaps, Benedict suggests, to the effect of age being offset by increase in body weight and improvement in physical condition.

Data on *elderly people* (60 years and over) have accumulated slowly. Reports have now been made on about 100 men in this age range by Benedict, by Lewis, by Hitchcock and Matson, by Boothby, Berkson, and Dunn, by Harris and Benedict, and by Aub and DuBois; and on about 75 women by Benedict and Meyer, by Hitchcock and Matson, by Boothby, Berkson and Dunn, by Harris and Benedict, and McKay and Patton. These results indicate that the basal metabolism decreases slightly as age advances; but more studies are needed at all ages between 60 and 100 years before satisfactory standards can be set up for this age range.

**Influence of sex.** Whether sex shall be said to influence the energy requirement will depend upon our use of terms. Benedict and Talbot found no difference in the basal metabolic rate of boys and girls of the same age until they reach a weight of 11 kilograms or a surface area of 0.48 square meter. Beyond a weight of 14 kilograms, boys maintain a persistently higher rate which is not apparently due at this early age (about 3 years) to greater muscular development.

Benedict and Emmes found, as previously noted, a slightly higher basal metabolism in men than in women of the same height and weight, but attribute this to a difference in the average composition of the body. See also the discussion of prediction formulas above.

Studies of the effect of menstruation on basal metabolism have not given consistent results. Not all women show an effect; in those who do, it does not always occur at the same part of the cycle; and in any case it is not large, different investigators reporting changes of from only 2 to 5 per cent. Recent reports are more consistent than the earlier studies in indicating a tendency to a premenstrual rise with a subsequent fall to a minimum level, during menstruation according to some, during the intermenstrual period according to others.

Root and Root made fortnightly determinations of the basal metabolism of a woman from the fourth month of pregnancy until the ninth week of lactation. Beginning with the sixth month, the metabolic rate rose steadily; on the eleventh day before delivery it was 7.6 per cent higher per kilogram of body weight than in the fourth month.

Sandiford and Wheeler made frequent determinations extending from a period previous to the establishment of pregnancy through the fourth month of lactation. Heat production just before delivery was 25 per cent higher than at the beginning of the study, while weight had increased 23 per cent. From an estimate of the surface area of the fetus, it was possible to calculate a quota of the total basal heat production of the pregnant woman which might be assigned to the metabolism of the fetus. The remainder might be considered to represent the heat production in the maternal body. This figure averaged 35.4 Calories per square meter per hour as compared with 34.7 Calories before pregnancy, a variation well within the limits of experimental error. Equally close agreement was shown by the data of other observers when recalculated by this method. In a general review, Harding concludes: "Pregnancy would thus appear to result in no alteration in the energy exchange beyond that produced by the growth within the maternal organism of a new mass of active protoplasmic tissue of a higher basal metabolic rate and comparable to that in infants."

Sandiford and Wheeler found no increase in basal heat production during lactation, and conclude that the conversion of the mother's food into milk does not involve a material loss of energy. But practical human experience as well as experiments upon farm animals supports the view that liberal increases in the food intake must be provided if optimal results in lactation are to be obtained.

**Influence of race.** A considerable number of studies of the basal metabolism of different races have now been made, the results being compared with our American standards in each case. The literature is excellently summarized by DuBois in his third edition (1936) of *Basal Metabolism in Health and Disease*. It may be said briefly here that there is evidence of some racial differences distinct from the effects of climate, diet, and exercise. Natives of Australia, Brazil, China, India, Java, the Philippines,

and Syria appear to have a lower basal metabolism, while the Japanese, Jamaicans, and white inhabitants of several Central and South American countries show little deviation from the usual American and European findings. Maya Indians in Yucatan and Araucanian Indians in Chile give evidence of a somewhat higher basal metabolism than the usual standards would predict for them.

**Influence of climate.** Studies on native whites in tropical and subtropical regions by de Almeida in Brazil, Sundstroem in tropical Australia, Tilt in Florida, Coons in Oklahoma, and Hafkesbring and Borgstrom in New Orleans indicate an average basal metabolism 10 to 20 per cent below that of whites living in a temperate climate.

**Influence of season.** Reports on seasonal fluctuations in the basal metabolism have been somewhat contradictory. Gustafson and Benedict, Hitchcock and Wardwell, and McKay report a tendency of the basal metabolism to be at its lowest level in the winter. On the other hand Griffith and his coworkers found it lowest in the late summer and early fall, and highest in winter or early spring. Hafkesbring and Collett also found it higher in cold than in hot weather.

**Influence of food.** Benedict and his associates studied the effect of a prolonged reduction in diet on basal metabolism. In a group of 12 college students an average loss of weight of 12 per cent was accompanied by an average lowering of the basal metabolic rate of 18 per cent per unit of body weight or of body surface. This finding supports the view that chronic undernutrition in adults, or even simple restriction of food consumption in health, if continued sufficiently, may bring the organism to a lower level of energy metabolism than would be indicated by the weight or surface. Underweight school children, as studied by Blunt, showed an increase in the basal rate of 16 to 25 per cent per kilogram of body weight. The striking contrast between the effects of undernutrition in the child as compared with the adult has not been satisfactorily explained.

No important difference has been found between the basal metabolic rate of vegetarians and non-vegetarians.

**Influence of habits of exercise.** After severe muscular activity basal metabolism is maintained at a higher level for a period proportionate to the amount of work done. There is also some

evidence of a relation between the degree of habitual exercise and the basal metabolic rate. A dog in Lusk's laboratory showed a remarkably uniform basal rate during the period when he was confined in a cage and a much higher metabolism on his return from an active vacation in the country.

Twelve white subjects living in Brazil had a basal metabolic rate 24 per cent lower than the accepted standards for a similar group in the United States. One of these men, all of whom led sedentary lives, entered upon a program of systematic exercise for a year and raised his basal metabolism from 22.7 to 32 Calories per square meter per hour.

**Influence of sleep.** Reports by Benedict, Benedict and Carpenter, and Mason and Benedict on the effect of sleep on the basal metabolism of adults are in fairly close agreement, indicating an average drop below the waking metabolism of about 10 per cent. Wang and Kern found a drop of 15 per cent in a group of boys and girls and Williams found 3- and 4-year-old children in close agreement, showing an average drop of about 16 per cent. Necheles noted less drop in his Chinese subjects than in his Westerners, which he thought might be due to the Chinese having lower muscle tone in the waking state than Westerners.

**Summary.** In a general review of the factors affecting normal basal metabolism Benedict concluded that "the basal metabolism of an individual is a function, first, of the total mass of active protoplasmic tissue, and, second, of the stimulus to cellular activity existing at the time the measurement of the metabolism was made." And that: "Perhaps the most striking factors causing variations in the stimulus to cellular activity are age, sleep, prolonged fasting, character of the diet, and the after effect of severe muscular work."

### Regulation of Body Temperature \*

Climate, season, housing, clothing, are all factors which may influence energy metabolism principally through their bearing upon the regulation of body temperature. It is evident that the maintenance of the body at a temperature above that of its ordinary environment involves a continual output of heat. This out-

\* For full discussion of the influence of surrounding temperatures upon metabolism and the relation of metabolism to the regulation of body temperature the reader is referred to Lusk's *Science of Nutrition*.

put of heat may be regulated in either of two ways: (1) By variations in the quantity of blood brought to the skin, which tend to control the loss of heat by radiation, conduction, and sweating; this is called "physical regulation." (2) By an increase in the rate of oxidation in the body in response to the stimulus of external cold; such a change in the rate of oxidation is known as "chemical regulation." The extra heat production which follows the taking of food (the specific dynamic action of the foodstuffs) may take the place of the "chemical regulation" and so help to protect the body from the necessity of burning material simply for the maintenance of its temperature. Muscular work, by increasing the production of heat in the body, may also render chemical regulation unnecessary; but apparently the specific dynamic action does not furnish energy which can be utilized for muscular work.

The presence of a layer of adipose tissue under the skin as well as the custom of covering the greater part of the external surface with clothing also tends to keep down the loss of heat to the point where "physical regulation" will suffice. Lusk cites experiments by Rubner upon a man whose metabolism was determined when kept in the same cold room but with different amounts of clothing, and observes that when the man was sufficiently clothed to be comfortable the "chemical regulation" was eliminated (*Science of Nutrition*, 4th edition, page 163).

In general it seems probable that people warmly clothed and living in houses which are heated in winter are not called upon to exercise "chemical regulation" to any considerable extent; in other words, they probably do not burn any considerable amount of material merely for the production of heat, the heat required for the maintenance of body temperature being obtained in connection with the metabolism which is essential to the maintenance of the muscular tension and the various other forms of internal work. If, however, the body be exposed to cold, it may be forced to employ "chemical regulation" with a resulting increase of the food requirement, and this will occur more readily in a thin person than in one who is well protected by subcutaneous fat.

The extra heat required in cold weather is probably obtained for the most part through the activities of the muscles. It is a matter of general experience that one instinctively exercises the muscles more vigorously in cold weather than in warm, and if one attempts to endure much cold without muscular exercise

there results shivering — a peculiar involuntary form of muscular activity whose function appears to be to increase heat production through increasing the internal work of the body.

To a large extent therefore the regulation of body temperature, in case of exposure to cold, is accomplished through the activity and tension of the muscles.

The foregoing discussion has reference primarily to adults. In the case of the infant whose surface is much greater in proportion to his weight and whose muscle tone is not yet fully developed, the loss of heat to the surroundings is not so readily checked by “physical” nor so easily made good by “chemical” regulation. Unless the infant is either warmly clothed or supplied with an artificial source of heat in cold weather he may be forced to burn, for warmth, material that might better be employed for growth.

### The Specific Dynamic Action of Foodstuffs

Atwater and Benedict determined directly by means of the respiration calorimeter the heat production of the same man during five fasting experiments of one to two days each, and during a four-day experiment with food about sufficient for maintenance. The average total metabolism on the fasting days was about 9 per cent lower than on the days when food was taken.

In longer fasts there may be a somewhat greater decrease in heat production. Thus, Benedict found that a man who weighed at the start 59.5 kilograms (131 pounds) metabolized, on the successive days of a seven-day fast, 1765, 1768, 1797, 1775, 1649, 1553, and 1568 Calories respectively. Naturally in long fasts factors other than the simple sparing of the direct effect of food come into play.\*

Tigerstedt studied by means of the carbon and nitrogen balance the metabolism of a man who fasted for five days and for the next two days took a very liberal diet. The data obtained are shown in Table 21.

These results show for man (as had previously been shown with dogs) that in fasting the total metabolism continues at a fairly constant rate in spite of the fact that the energy is obtained entirely at the expense of the body material. In this case, the diet

\* For a detailed account of the results obtained in a fasting experiment of 31 days' duration, see Benedict, *A Study of Prolonged Fasting*, Publication No. 203 of the Carnegie Institution of Washington.



TABLE 21. — DATA OF TIGERSTEDT'S FASTING AND REFEEDING EXPERIMENT

|                                       | BODY WEIGHT<br>KILOS | CALCULATED<br>TOTAL<br>METABOLISM<br>CALORIES | CALORIES PER<br>KILO |
|---------------------------------------|----------------------|---|----------------------|
| 1st fast day . . . . .                | 67.0                 | 2220 <sup>a</sup>                             | 33.2 <sup>a</sup>    |
| 2nd fast day . . . . .                | 65.7                 | 2102 <sup>a</sup>                             | 32.0 <sup>a</sup>    |
| 3rd fast day . . . . .                | 64.9                 | 2024  | 31.2                 |
| 4th fast day . . . . .                | 64.0                 | 1992  | 31.1                 |
| 5th fast day . . . . .                | 63.1                 | 1970  | 31.2                 |
| Fed 4141 Calories . . . . .           | 64.0                 | 2437  | 38.1                 |
| Fed 4141 Calories (2nd day) . . . . . | 65.6                 | 2410  | 36.8                 |

<sup>a</sup> These figures are slightly too high because the loss of carbon on these days was due in part to combustion of glycogen, but is calculated as if due simply to protein and fat.

given at the end of the fasting period (4141 Calories) was approximately double what would have been required for maintenance, but the increase in energy metabolism was only 22.5 per cent over that of fasting.

The results of fasting experiments thus make it evident that the body has but little power in the direction of adjusting its energy metabolism to the energy value of its food supply.

Rubner found that each type of food exerted a more or less specific influence upon the energy metabolism, so that when the foodstuffs were fed separately, somewhat different energy values were required for the maintenance of body equilibrium. Thus, if the total metabolism of a dog fasting at 33° C. be represented by 100 Calories, he must be fed, in order to prevent loss of body substance, about 106.5 Calories of sugar, or 114.5 Calories of fat, or 140 Calories of protein. Lusk, however, has reported as average typical results of experiments with dogs in his laboratory 106 Calories of glucose, 104 Calories of fat, or 130 Calories of meat protein, indicating that the specific dynamic action of fat is less than that of carbohydrate rather than more as Rubner reported. A man observed by Rubner metabolized in fasting 2042 Calories; when fed 2450 Calories in the form of sugar alone, he metabolized 2087 Calories; when fed 2450 Calories in the form of meat alone, he metabolized 2566 Calories.

Lusk and his coworkers investigated this influence of the foodstuffs upon metabolism, called the specific dynamic action, very extensively and developed the subject to such an extent that for an adequate discussion of their results the original articles in

the *Journal of Biological Chemistry* or Lusk's own summaries \* should be consulted.

In his last review of this subject Lusk concluded that the evidence at hand supported the view that the increased heat production which follows the influx of fat into the blood is due simply to the increased concentration of oxidizable material, whereas that which follows ingestion of carbohydrate is due to the heat of intermediary reactions between glucose and glycogen, and that following ingestion of protein to the stimulating action of some of its products of digestion and intermediary metabolism. In a more recent review of the subject Wilhelmj (1935) shows that these explanations of the specific dynamic action of fat and carbohydrate are still the best that can be given, but cites evidence to support the view that the specific dynamic action of protein represents the energy of the process of deaminization of the amino acids and formation of urea and that it might best be expressed as the Calories of extra heat per millimol of amino acid deaminized.

On an ordinary mixed diet, however, this apparent loss of energy due to eating protein is not a very large factor in the total metabolism, since the total specific dynamic action makes the metabolism of energy for the day probably less than one tenth higher on a full maintenance ration than when no food is eaten. DuBois concludes that on an ordinary mixed diet an allowance of 6 per cent of the Calories of the food is doubtless a safe amount.

It is sometimes thought that superior preparation or very thorough mastication of food results in such improvement in its utilization that a material saving may be effected in the amount of food required. But it will be remembered that under average conditions only about 5 per cent of the energy value of the food is lost in digestion or expended upon the digestive process. Any improvement in those conditions through superior preparation or mastication of the food can therefore at most effect a saving of less than 5 per cent of the energy value. Thus the influence upon total food requirement is scarcely appreciable. The advantages of good preparation and thorough chewing of the food are very important, but they lie in other directions than reduction in the amount of food required.

\* Lusk. *Medicine* 1, page 311 (1922). *Science of Nutrition*, Chapter XII. *Journal of Nutrition* 3, page 519 (1931).

The adjustment of total food energy intake to the activities of everyday life is considered in the next chapter.

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## CHAPTER X

### TOTAL ENERGY METABOLISM AND FOOD REQUIREMENT

#### Influence of Muscular Work

MUSCULAR work is much the most important of the factors which raise the food requirements of adults above the basal rate necessary for mere maintenance.

Accurate measurements by means of the calorimeter have shown that the average total metabolism of a man sitting still is about 100 Calories per hour; while the same man working actively increases his metabolism up to about 300 Calories per hour; and a well-trained man working at his maximum capacity metabolizes material enough to liberate 600 Calories per hour, *i.e.*, his metabolism may be six times as active during the hours actually spent in such work as when he is at rest. If during 24 hours a man works as hard as this for 8 hours and spends 2 hours in such light exercise as going to and from work, his food requirement for the day will be somewhat over 6000 Calories, or three times the maintenance requirement. Thus, work may increase the day's metabolism as much as 200 per cent, whereas liberal feeding at the end of a fast was found to increase the metabolism only 22.5 per cent, or one ninth as much. Only a few exceptional occupations, such as that of lumbermen, for example, involve such heavy work as to cause a metabolism of 6000 Calories per day. More often the man who works eight hours a day at manual labor will increase his metabolism by 1000 to 2000 Calories above what is needed for maintenance at rest, making his total food requirement 3000 to 4000 Calories.

Voit estimated the food requirement of a "moderate worker" at about 3050 Calories; and Atwater, in adapting this standard to American conditions, increased the allowance to 3400-3500 Calories in the belief that the American works more rapidly and therefore with a greater expenditure of energy. The mistake is

often made of supposing that these estimates were intended for everyone who leads an active life, whereas they really contemplate a long day of manual labor, for Voit's definition of "moderate worker" was a man laboring 9 or 10 hours a day at an occupation such as that of a carpenter, mason, or joiner.

The amount of energy spent during 24 hours by a sedentary worker will depend not only upon the number of hours which he devotes to exercise, but especially upon the kind of exercise chosen. Lusk estimates that an average-sized man sleeping 8 hours, sitting 14 hours, and walking 2 hours spends about 2500 Calories; whereas if he spends 2 hours in vigorous exercise instead of walking, his total energy output rises to about 3000 Calories.

The importance of muscular activity as the chief factor governing the energy expenditure and food requirement of healthy adults calls for a careful quantitative study of its effect upon metabolism.

**Quantitative relation between work performed and total metabolism.** Theoretically it is possible to determine the mechanical efficiency of a man by dividing the mechanical effect of his work by the increase of energy metabolism which the work involves. This gives the basis on which to ascertain how much extra food would be necessary to supply the energy required for the performance of any given task.

Zuntz and his associates in Berlin carried out a long series of experiments of this kind which were described by Magnus-Levy in Von Noorden's *Metabolism and Practical Medicine*.

Benedict and others have studied the mechanical efficiency of both men and women in level and grade walking. These studies indicate that in level walking the efficiency is highest at moderate speeds. Very fast walking (about  $5\frac{1}{2}$  miles per hour) required a greater heat output than running at the same speed. In grade walking the expenditure for a given amount of work tended to be less at low speed on a high grade than at high speed on a low grade. The greatest net efficiency in grade walking (approximately 30 per cent) was reached only when the work done was less than 500 kilogrammeters per minute.

From the data obtained from these and similar studies on walking it is possible to estimate roughly the food requirements of men who neither do active physical labor nor take vigorous exercise, yet move about more freely than in the so-called rest experi-

ments already described. If, for example, it be assumed that a healthy man would require 2200 Calories per day when remaining in one room, and that the total additional muscular movements of a day at business and recreation were equivalent to walking five miles on level ground, his total food requirement for the day would become 2500 to 2700 Calories (36 to 39 Calories per kilogram), while activity equivalent to walking ten miles on level ground would bring the total daily requirements to 2800 to 3200 Calories (40 to 46 Calories per kilogram).

By means of the respiration calorimeter, Atwater and Benedict studied the question of mechanical efficiency with a different form of muscular work. They placed in the calorimeter chamber an ergometer, which consisted of a fixed bicycle frame having in place of the rear wheel a metal disk which revolved against a measured amount of electrical resistance, so that the mechanical effect of the muscular work was very accurately determined. The expenditure of energy involved in the performance of this work was estimated by comparing the total metabolism of a working day with that of the same man when living in the calorimeter chamber at rest. The average results obtained with three different men were as shown in Table 22.

TABLE 22. — ATWATER AND BENEDICT DATA ON MECHANICAL EFFICIENCY OF MAN

| SUBJECT AND NATURE OF EXPERIMENT                | ENERGY TRANSFORMED |                          | HEAT EQUIVALENT OF WORK PERFORMED | MECHANICAL EFFICIENCY |
|---|--------------------|--------------------------|-----------------------------------|-----------------------|
|   | Total per Day      | Excess over That at Rest |                                   |                       |
|   | <i>Calories</i>    | <i>Calories</i>          | <i>Calories</i>                   | <i>per cent</i>       |
| Subject E. O.                                   |                    |                          |                                   |                       |
| Average 13 rest experiments (42 days) . . . . . | 2279               |                          |                                   |                       |
| Average 3 work experiments (12 days) . . . . .  | 3892               | 1613                     | 214                               | 13.3                  |
| Subject J. F. S.                                |                    |                          |                                   |                       |
| Average 4 rest experiments (12 days) . . . . .  | 2119               |                          |                                   |                       |
| Average 6 work experiments (18 days) . . . . .  | 3559               | 1440                     | 233                               | 16.2                  |
| Subject J. C. W.                                |                    |                          |                                   |                       |
| Average 1 rest experiment (4 days) . . . . .    | 2357               |                          |                                   |                       |
| Average 14 work experiments (46 days) . . . . . | 5143               | 2786                     | 546                               | 19.6                  |

With an improved ergometer of the same type as that used in the experiments just cited, Benedict and Carpenter working with J. C. W. (one of the 3 men above mentioned) found efficiencies ranging from 20.7 to 22.1 per cent and averaging 21.6 per cent; with other men studied, the efficiencies ranged from 18.1 to 21.2 per cent.

Benedict and Cathcart, in similar bicycle ergometer experiments in which the basis of comparison was complete rest on a couch, found efficiencies varying from 10 to 25 per cent, depending on load, speed, and the familiarity of the subject with the work, the maxima for the six men studied being 23.1, 20.4, 21.6, 22.7, 20.8, and 25.2 per cent respectively.

In another series of experiments they subtracted from the expenditure of energy during work, the amount spent when the subject, instead of lying on a couch, sat on the ergometer and allowed the pedals to be turned under his feet. Using this method of estimation they were able by careful adjustment of speed and load to realize with a professional bicycle rider an efficiency of 33 per cent or as much as Zuntz and his associates had estimated from the walking experiments.

Only under the most favorable circumstances and with subjects fully accustomed to the kind of work being performed will the actual mechanical effect produced amount to as much as one fourth to one third of the extra energy expended during work over that during rest, *i.e.*, to an efficiency of 25 to 33 per cent. Not only do most occupations involve kinds of work which in their nature must be done with less efficiency than walking (or riding a stationary ergometer) but the usual hours of labor are longer than those in which the maximum mechanical efficiency is attained. The efficiency may begin to decline before any sensation of fatigue is felt.

Thus Leo Zuntz found, when he rode his bicycle for four successive hours at an average rate of 15 to 17 kilometers (about 9 miles) per hour, that he experienced no feeling of fatigue, but his determinations showed that the expenditure of energy necessary to produce a given effect had increased about 9, 13, 10, and 23 per cent at the end of 1, 2, 3, and 4 hours respectively. This is because if the same kind of work be performed for a series of hours, auxiliary muscles are gradually brought increasingly into action, partly for the performance of the work itself, partly for the fixa-

tion of the bodily framework (maintenance of posture). These auxiliary muscles work less economically than those which are used first and most naturally. For much the same reasons there is a lower efficiency in the case of work which is from the first of too fatiguing a nature because of being either excessive or unsuitably distributed. When Leo Zuntz increased his speed 2.4 times, he found his metabolism increased 4.3 times, implying a considerable loss of efficiency. Under the conditions of Benedict and Cathcart's experiments also the efficiency was usually decreased upon increasing the speed; on the other hand a moderately heavy load was more economical than a light one.

### Total Energy Requirement of Adults

It is now possible to estimate the approximate average expenditure of energy per hour under a considerable number of conditions of muscular activity. For convenience of comparison and application the original data have been reduced to a common basis of 70 kilograms (154 pounds), with the results shown in Table 23, compiled by Professor M. S. Rose.

By the use of these estimates the probable food requirement for a person of 70 kilograms (154 pounds) may be calculated very simply, as, for instance, in the following example:

|   |                      |
|---|----------------------|
| 8 hours of sleep at 65 Calories           | = 520 Calories       |
| 2 hours' light exercise * at 170 Calories | = 340 Calories       |
| 8 hours' carpenter work at 240 Calories   | = 1920 Calories      |
| 6 hours' sitting at rest at 100 Calories  | = 600 Calories       |
| Total food requirement for the day,       | <u>3380 Calories</u> |

Tigerstedt, in his *Textbook of Physiology*, gives the following estimates of food requirements for different degrees of activity as indicated by means of typical occupations, which may be useful in checking results calculated as above:

- 2000-2400 Calories per day suffice for a shoemaker.
- 2400-2700 Calories per day suffice for a weaver.
- 2700-3200 Calories per day suffice for a carpenter or mason.
- 3200-4100 Calories per day suffice for a farm laborer.
- 4100-5000 Calories per day suffice for an excavator.
- Over 5000 Calories per day are required by a lumberman.

\* Going to and from work, for example.

TABLE 23. — ENERGY EXPENDITURE PER HOUR UNDER DIFFERENT CONDITIONS OF MUSCULAR ACTIVITY

| FORM OF ACTIVITY   | CALORIES PER HOUR                  |              |           |
|--|------------------------------------|--------------|-----------|
|  | Per 70 Kilo-grams<br>(Average Man) | Per Kilogram | Per Pound |
| Sleeping . . . . .                                       | 65                                 | 0.93         | 0.43      |
| Awake lying still . . . . .                              | 77                                 | 1.10         | 0.50      |
| Sitting at rest . . . . .                                | 100                                | 1.43         | 0.65      |
| Reading aloud . . . . .                                  | 105                                | 1.50         | 0.69      |
| Standing relaxed . . . . .                               | 105                                | 1.50         | 0.69      |
| Hand sewing . . . . .                                    | 111                                | 1.59         | 0.72      |
| Standing at attention . . . . .                          | 115                                | 1.63         | 0.74      |
| Knitting (23 stitches per minute on sweater) . . . . .   | 116                                | 1.66         | 0.75      |
| Dressing and undressing . . . . .                        | 118                                | 1.69         | 0.77      |
| Singing . . . . .  | 122                                | 1.74         | 0.79      |
| Tailoring * . . . . .                                    | 135                                | 1.93         | 0.88      |
| Typewriting rapidly . . . . .                            | 140                                | 2.00         | 0.91      |
| Ironing (with five-pound iron) . . . . .                 | 144                                | 2.06         | 0.93      |
| Dishwashing (plates, bowls, cups, and saucers) . . . . . | 144                                | 2.06         | 0.93      |
| Sweeping bare floor (38 strokes per minute) . . . . .    | 169                                | 2.41         | 1.09      |
| Bookbinding . . . . .                                    | 170                                | 2.43         | 1.10      |
| "Light exercise" . . . . .                               | 170                                | 2.43         | 1.10      |
| Shoe making . . . . .                                    | 180                                | 2.57         | 1.17      |
| Walking slowly (2.6 miles per hour) . . . . .            | 200                                | 2.86         | 1.30      |
| Carpentry, metal working, industrial painting . . . . .  | 240                                | 3.43         | 1.56      |
| "Active exercise" . . . . .                              | 290                                | 4.14         | 1.88      |
| Walking moderately fast (3.75 miles per hour) . . . . .  | 300                                | 4.28         | 1.95      |
| Walking down stairs . . . . .                            | 364                                | 5.20         | 2.36      |
| Stoneworking . . . . .                                   | 400                                | 5.71         | 2.60      |
| "Severe exercise" . . . . .                              | 450                                | 6.43         | 2.92      |
| Sawing wood . . . . .                                    | 480                                | 6.86         | 3.12      |
| Swimming . . . . .                                       | 500                                | 7.14         | 3.25      |
| Running (5.3 miles per hour) . . . . .                   | 570                                | 8.14         | 3.70      |
| "Very severe exercise" . . . . .                         | 600                                | 8.57         | 3.90      |
| Walking very fast (5.3 miles per hour) . . . . .         | 650                                | 9.28         | 4.22      |
| Walking up stairs . . . . .                              | 1100                               | 15.8         | 7.18      |

Lusk gives the following summary of energy requirements of women at work at typical occupations as investigated by Becker and Hamäläinen in Finland:

A seamstress sewing with a needle required 1800 Calories.

Two seamstresses, using a sewing machine, required 1900 and 2100 Calories, respectively.

Two bookbinders required 1900 and 2100 Calories.

Two household servants, employed in such occupations as cleaning windows and floors, scouring knives, forks, and spoons, scouring copper and iron pots, required 2300 to 2900 Calories.

Two washerwomen, the same servants as the last named, required 2600 and 3400 Calories in the fulfillment of their daily work.

### **Energy Requirements for Pregnancy and Lactation**

These factors have been discussed in relation to the basal metabolism in Chapter IX.

Although the basal metabolism does not seem to be greatly increased per unit of weight in pregnancy, yet by virtue of her increase in weight the pregnant woman has a larger total energy requirement. The effect of increased weight is likely to be offset to some extent by the decrease in activity. During lactation, when the entire nutritive requirement of the nursing infant is being met through the mother, the energy needs of the latter are greatly increased. Production of milk involves an extra energy requirement beyond the actual energy value of the milk secreted, and the food allowance should provide for it accordingly. Liberal feeding of the nursing mother (*e.g.*, up to 2800 to 3000 Calories for a woman with moderate muscular exercise) is not only important for the conservation of her own bodily resources but may prolong the period of lactation and thus be of great value to the child as well.

### **Total Energy Requirements of Children**

The relatively large energy requirement of children as compared with that of adults is due to a higher basal metabolism, vigorous muscular activity, and stimulation from their food intake, which must be liberal to support the storage of protein and fatty material in the body for growth.

The cost of activity in children has not been measured very extensively as yet. In studies of infants, Benedict and Talbot found that crying could increase the energy output as much as 200 per cent above the basal metabolism although the average

increase was only about 65 per cent; while Murlin and his coworkers found an increase of 100 per cent but came to the conclusion that 30 per cent is probably sufficient allowance to make for the average amount of crying by the normal infant. In another study Benedict and Talbot reported that while vigorous crying and kicking may increase heat production by 50 per cent or more, the average increase for 24 hours may not be more than 25 per cent. Older children were studied by Sondén and Tigerstedt, who found that in school boys and girls sitting quietly in a respiration chamber the heat production was at a level 75 and 50 per cent respectively above the basal level. Bedale measured the energy cost of several activities of boys and girls in an English private school and found increases of 56 to 760 per cent above the basal metabolism, according to the kind of activity. Helmreich found that the exercise of lifting the legs fifteen times a minute increased the energy metabolism of the younger children 20 to 25 per cent but that in the older children with longer legs the increase amounted to as much as 80 to 100 per cent. McClintock and Paisley found that the cost of horizontal walking in 65 boys was 20 per cent greater than that of the same exercise in men. Potgieter, measuring the oxygen consumption (or carbon dioxide production) of 9- and 10-year-old girls standing still, at quiet play, and climbing stairs, found elevations above the basal metabolism of 31, 90, and 290 per cent respectively. Robb measured the energy expended during quiet play by seven 3- and 4-year-old children (4 girls and 3 boys) and found it averaged 66 and 69 per cent above the basal metabolism for the girls and boys respectively. Taylor, working with six 9- to 11-year-old boys, found an average expenditure above the basal metabolism of 53 per cent for quiet play and of 212 per cent for bicycle riding which was performed with a gross efficiency of 15.8 per cent or a net efficiency above the basal metabolism of 23.1 per cent. In girls in the same age range Thompson found an average expenditure above the basal metabolism of 71 per cent for quiet play and of 250 per cent for bicycling which was performed with a gross efficiency of 13.9 per cent or a net efficiency above the basal metabolism of 19.3 per cent. (See references at the end of Chapter IX.)

About the stimulating influence of food upon metabolism during growth very little is known but it appears to be true that food stored in the growing body does not stimulate metabolism and



that under ordinary conditions 6 per cent of the total energy intake is a safe allowance to make for this.

With normal adults the energy requirement is generally considered to be approximately equal to the energy expenditure. With children, on the other hand, the energy requirement includes an additional provision for growth. In studies of infants 7 to 9 months old, Rubner and Heubner found a storage of 12.2 per cent of the energy value of the food consumed, and Camerer found a storage of 15 per cent of the energy and 40 per cent of the protein of the diet. An allowance of 10 to 15 per cent of the calories of the basal metabolism is generally considered a safe allowance for storage except in the periods of most rapid growth.

In order to provide adequately for all contingencies and support the rapid growth which is normal at this age, it is estimated that a vigorous child will require during the greater part of the first year about 100 Calories of food per kilogram of his body weight per day. But in cases of artificial feeding, since the digestive tract must be gradually educated to handle the milk of a different species, it will often be necessary to feed much less than 100 Calories per kilogram per day at first, perhaps for several months, and only very gradually increase the food allowance.

From the end of the first year until growth is completed the food requirement increases, but not so rapidly as does the body weight, so that while the allowance of food becomes larger per day it becomes smaller per kilogram.

Tables giving the energy requirement at different ages may be found in Rose's *Laboratory Handbook for Dietetics* and in Chapter XXVII beyond.

Children who are very active or growing very rapidly may require even more food than these tables suggest. Such cases are perhaps most frequently found among boys between 10 and 15 years of age. DuBois finds in boys 12 and 13 years old an average *basal* metabolism (complete rest and almost complete fasting) of 1.76 Calories per kilogram per hour, or about 75 per cent above that of healthy adults.\* Assuming average activity for boys of this age the energy expenditure during 24 hours would probably amount to 60 to 70 Calories per kilogram and as this is

\* Per unit of surface the basal energy metabolism of these boys was about 25 per cent higher than that of healthy men.

a period of rapid growth the *requirement* would be materially higher than the rate of expenditure.

In the White House Conference Reports of 1932 Roberts presented the analysis of data on the energy intake of 220 girls and 253 boys from 2 to 17 and 18 years respectively. The conclusion was reached that for girls 2 to 17 years of age there is a close correlation between the total energy requirement and height; and that for moderately active girls 6 to 16 years of age 39 to 40 Calories per inch should be allowed, while before 6 and after 16 years the allowance should be 35 to 37 Calories per inch. For boys the Calories per inch seem to increase with age from 35 at 2 years to 55 at 17 years. These allowances are for moderately active girls and boys. Larger allowances must be made for more active children on whom more data are needed at all ages.

### • Food Calories and the Control of Body Weight

While the body's weight may sometimes be significantly influenced by its water balance, in the vast majority of cases overweight means over-fatness.

We have seen that surplus calories of ingested food, whether taken in the form of protein, fat, or carbohydrate, tend to accumulate in the form of body fat. And over-fatness always means that the intake of food calories has been out of proportion to the expenditure of energy *by the person concerned*. Undoubtedly there is more tendency to over-weight in some people than in others, and undoubtedly, too, this sometimes extends beyond the question of appetite to endocrine and perhaps other constitutional differences; yet the fact remains that for the individual the control of body weight is essentially a matter of a proper balance between what is ingested as food and what is oxidized in the energy metabolism. If one tends to become too fat, the remedy is to eat less or to burn more.

To increase basal metabolism by administration of thyroid, thyroxine, or any artificial drug designed to increase oxidation is too dangerous for any except very rare cases under strict medical control.

To increase the energy metabolism by muscular exercise is probably beneficial in a fair proportion of cases but is usually a much slower process of weight reduction than one desires, especially as exercise usually increases the appetite. It is also to be kept in mind that strenuous exercises may be dangerous to people who

are over-weight or no longer young. Such people may well remember the principle so ably expounded by M. S. Rose that the only form of exercise essential to the control of body weight is the exercise of the intelligence.

Intelligent observation indicates clearly that for the greater number of people the problem is more readily solved by concentrating attention upon control of food calorie intake rather than upon increase of metabolism. The scientific attitude does not simply ask, Is this food fattening?, nor even simply, How fattening is it?, but *both*, How does it stand as a source of calories?, and, How important is it as a source of protein and of the needed mineral elements and vitamins? If a slogan must be carried in mind, perhaps, "No calories without vitamins," is as good a precept as any.

Almost always there is room enough for the desirable degree of reduction of calorie intake among the foods that are not important sources of mineral elements and vitamins, and it is among such foods that the cutting out or the cutting down should be done. Several of the chapters beyond bear directly upon this part of the problem.

Where an actual reduction of body weight is sought, one should not proceed too drastically, nor should one be discouraged or confused by "short-range" fluctuations. Not only may the body gain or lose a few pounds of water without apparent reason or effect upon health, but also the fluctuations in the body's glycogen influence its water content also, each gram of glycogen being usually accompanied by about three grams of water. Thus 400 Calories gained or spent in the form of 100 grams of glycogen may quickly change the body weight about 400 grams, while this weight gained or lost as fat would involve about 3600 Calories.

Keeping in mind therefore that sudden fluctuations of a few pounds do not call for any change of plan, and that any weight-reduction plan should aim not at spectacular results but at steady moderate progress, it is well to deduct about 500 Calories from the daily intake which would keep the body weight constant.

This withdrawal from bodily stores of 500 Calories per day or 3500 Calories per week will normally mean the burning-off of about 380 grams of actual fat. And this will mean a reduction of body weight by about one pound of adipose tissue; for adipose tissue is about four fifths actual fat and about one fifth water.

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## CHAPTER XI

### QUANTITATIVE ASPECTS OF PROTEIN NEEDS AND VALUES

THE MORE qualitative aspects of protein metabolism, the relation of individual amino acids to the meeting of nutritional needs, and the fact that food proteins differ in their amino-acid constitution, have been discussed in earlier chapters.

The purpose of the present chapter is to consider the closely connected quantitative problems of the amounts of protein needed for normal nutrition and the relative nutritional values ("biological efficiencies") of the protein-mixtures of different foods.

#### Protein Metabolism in Fasting

As the nature and amount of food consumed have a great influence upon the rate at which protein is metabolized in the body, one's first thought might be to seek something like a basal rate of protein metabolism in fasting. But in fasting the energy metabolism is not greatly lowered and so the body must burn as fuel considerable amounts of the substances which it contains; hence there is a likelihood that in fasting some protein may be used simply as fuel, which would result in an over-estimate of the true protein minimum.

Moreover, actual observations have shown that the rate of protein metabolism in fasting is considerably influenced by the previous habit as to the daily amounts of protein consumed and metabolized, and very considerably also by the stores of glycogen and fat available in the body for its use as fuel.

For such reasons, the nitrogen output during fasting, while often supplying data of much value in other connections, does not show us anything approximating a constant base-line for the study of the needs of normal nutrition. The presentation and discussion of numerical data regarding fasting is therefore postponed until the next chapter, where it will be of interest in connection with the corresponding data for the so-called mineral elements.

As a means of approach to the normal protein requirement, we can here more advantageously study the data of experiments designed to determine how much protein must be consumed in order to keep the adult body in protein (or nitrogen) equilibrium.

As explained in Chapter VIII, quantitative determinations of nitrogen intake and output are taken as measures of the amounts of protein received in the food and metabolized in the body, figures for nitrogen being multiplied by 6.25 to obtain corresponding figures for protein. This involves the two assumptions that the food contains only negligible amounts of nitrogen in forms other than protein, and that the proteins have an average nitrogen content of 16 per cent. Each of these assumptions might be criticized in detail; but the errors involved are usually not great, especially in studies of human nutrition.

#### **Nitrogen Balance Experiments and the Tendency toward Equilibrium at Different Levels of Protein Intake**

The determination of the nitrogen balance has already been mentioned as one factor in the study of the total food requirement by means of metabolism experiments; and it has been shown that the *balance* is found by subtracting the output from the intake. (Theoretically the elimination through the skin should be determined and included in the calculation; practically, this is usually neglected unless, on account of warm weather or vigorous exercise, the subject perspires profusely.) When intake exceeds output, there is a plus balance which indicates a storage of nitrogen and therefore presumably of protein in the body; a minus balance (greater output than intake) indicates a loss of body protein. When the balance is 0, or so near 0 as to be within the limits of experimental error, the body is said to be *in nitrogen (or protein) equilibrium*.

The healthy full-grown body tends to establish nitrogen equilibrium by adjusting its rate of protein metabolism to its food supply within wide limits. The time required by the body for this adjustment depends mainly upon the extent to which the diet is changed.

The following observations by Von Noorden illustrate the establishment of equilibrium after only moderate changes in the diet:

(1) A young woman weighing 58 kilograms (128 pounds) at rest in bed was given food furnishing 1860 Calories per day (Table 24).

TABLE 24. — EXAMPLE OF ADJUSTMENT TO DIMINISHED INTAKE

|   |             |
|---|-------------|
| Total nitrogen of food per day . . . . .                        | 16.96 grams |
| Lost in digestion (average nitrogen in feces) per day . . . . . | .94 gram    |
| "Absorbed" per day (average) . . . . .                          | 16.02 grams |

|                   | NITROGEN ELIMINATED<br>THROUGH KIDNEYS | NITROGEN BALANCE |
|-------------------|--|------------------|
|                   | grams                                  | grams            |
| 1st day . . . . . | 18.2                                   | — 2.18           |
| 2nd day . . . . . | 17.0                                   | — 0.98           |
| 3rd day . . . . . | 15.8                                   | + 0.22           |
| 4th day . . . . . | 16.0                                   | + 0.02           |
| 5th day . . . . . | 15.7                                   | + 0.32           |

Here there was practical equilibrium after the second day. This was a case of *adjustment to a lowered protein intake*, for the food previously taken was known to be rich in protein.

(2) Another experiment was made by Von Noorden with the same person to show the time required to reach equilibrium *after increasing the intake of protein*. In this case the food furnished 2030 Calories per day and the nitrogen balance was as shown in Table 25.

TABLE 25. — EXAMPLE OF ADJUSTMENT TO INCREASED INTAKE

| DAY | NITROGEN<br>IN FOOD | NITROGEN<br>IN FECES | NITROGEN<br>ABSORBED | NITROGEN<br>IN URINE | NITROGEN<br>BALANCE |
|-----|---------------------|----------------------|----------------------|----------------------|---------------------|
|     | grams               | grams                | grams                | grams                | grams               |
| 1   | 14.40               | 0.70                 | 13.70                | 13.60                | + 0.10              |
| 2   | 14.40               | 0.70                 | 13.70                | 13.80                | — 0.10              |
| 3   | 14.40               | 0.70                 | 13.70                | 13.60                | + 0.10              |
| 4   | 20.96               | 0.82                 | 20.14                | 16.80                | + 3.34              |
| 5   | 20.96               | 0.82                 | 20.14                | 18.20                | + 1.94              |
| 6   | 20.96               | 0.82                 | 20.14                | 19.50                | + 0.64              |
| 7   | 20.96               | 0.82                 | 20.14                | 20.00                | + 0.14              |

Here, where the amount of protein fed was increased from 90 to 130 grams without change in the total fuel value of the diet, the body reached equilibrium on the fourth day after the increase.

It is apparent, therefore:

(1) That the body tends to adjust its protein metabolism to its protein supply.

(2) That when the body is accustomed to a certain rate of



protein metabolism, it requires an appreciable length of time to adjust itself to a materially higher or lower rate.

Hence the rate of protein metabolism on any given day will depend in part upon the rate of metabolism to which the body has been accustomed and in part upon the protein intake for the day. When the protein supply varies from day to day, the metabolism for each day is influenced by both the factors, with the net result that the elimination approximately equals the intake when averaged for a sufficiently long period, although the data for any particular day might show a distinct gain or loss. When the protein supply is constant for a few days, the effect of previous habit usually disappears and approximate equilibrium is established as in the above cases.

A transitory period of loss of nitrogen from the body is apt to be due simply to taking less than the usual amount of protein food; but when the body loses nitrogen for many days in succession it becomes probable that the diet is insufficient, either in total calories or in protein, to enable the usual adjustment to take place.

A transitory period of storage of nitrogen in the body may occur as the result of an increase either of the protein or of the total fuel value of the food; but a persistent storage occurs, as Von Noorden has pointed out, only under the following conditions:

- (1) In the growing body (or in pregnancy) where new tissue is being constructed;

- (2) In cases where increased muscular exercise results in enlargement of the muscles;

- (3) In cases where, owing to previous insufficient feeding or to wasting disease, the protein content of the body has been more or less diminished and consequently any surplus available is utilized to make good the loss.

### **Protein-Sparing Action of Carbohydrates and Fats**

It has been shown that in fasting experiments the stored glycogen and fat in the body exert a "sparing" influence upon protein metabolism, the amount of protein catabolized being smaller when the supplies of glycogen and fat are more abundant. Similarly the amounts of carbohydrates and fats in the food influence the rate of protein metabolism as indicated by the nitrogen excretion. The loss of protein which occurs on an insufficient diet may

be diminished or even stopped by adding carbohydrate or fat to the food; and if carbohydrate or fat be added to the diet of a man in nitrogen equilibrium, there results a temporary decrease in nitrogen output with a corresponding storage of protein in the body. If the former observation stood alone, it could be interpreted as meaning simply that the body draws upon its stored protein for energy so long, and only so long, as the fuel value of the food is insufficient; but the fact that addition of carbohydrate or fat to a diet already sufficient may cause an actual storage of protein indicates that the "protein-sparing action" or "protein-protecting power" of carbohydrates and fats involves something more than merely the question whether the body "needs" to burn protein as fuel.

As this is a matter of great importance, it may be well to consider somewhat carefully (1) some of the experimental evidence, and (2) a theoretical explanation regarding the protein-sparing action of the carbohydrates and fats. For an account of the earlier experiments on this subject, especially those of Voit and Rubner upon dogs, the reader is referred to Lusk's *Science of Nutrition*. Only some typical experiments upon men can be described here.

Lusk (1890), experimenting upon himself, showed the susceptibility of the protein metabolism to the sudden withdrawal of carbohydrate food. In a typical experiment, withdrawal of carbohydrate increased the nitrogen excretion from 11.44 to 17.18 grams per day.

Kayser compared the efficiency of carbohydrates and fats as spacers of protein by observing the effect upon the nitrogen balance of replacing the carbohydrates of the food by such an amount of fat as would furnish the same number of calories, and then, after three days, resuming the original diet. This experiment and that of Tallquist which follows are given somewhat fully, as they illustrate well the methods and results of investigations based mainly upon the question of nitrogen equilibrium. Kayser, who served as his own subject, was twenty-three years old, of good physique, with a small store of body fat, and weighed 67 kilograms. In the first and third periods he ate meat, rice, butter, cakes, sugar, oil, vinegar, and salad. In the second period the diet was changed so as to consist of meat, eggs, oil, vinegar, and salad, so that nearly all of the digestible carbohydrate was withdrawn and replaced by fat. The two diets had practically the same fuel value and protein content. The results of this experiment are shown in Table 26.

It is evident from the nitrogen balance of the first period that the amount of protein in the food was here greater than necessary, but that equilibrium was established in four days. On substituting fat for carbohydrate (calorie for calorie) there was a marked increase of nitrogen output and, what is especially noteworthy, there was no evidence of any tendency

to regain equilibrium during this period, but on the contrary the loss of nitrogen became greater each day the fat diet was continued; whereas, upon returning to the mixed diet, not only was the loss of protein stopped, but the body almost at once began replacing the protein it had lost, although the protein and energy values of the food were practically unchanged.

TABLE 26. — NITROGEN BALANCE WHEN FEEDING ISODYNAMIC QUANTITIES OF CARBOHYDRATE AND FAT (Kayser)

| PERIOD | DAY | INTAKE         |              |                    |                 | OUTPUT         | NITROGEN BALANCE |
|--------|-----|----------------|--------------|--------------------|-----------------|----------------|------------------|
|        |     | Total Nitrogen | Fat          | Carbohy-<br>drates | Fuel Value      | Total Nitrogen |                  |
|        |     | <i>grams</i>   | <i>grams</i> | <i>grams</i>       | <i>Calories</i> | <i>grams</i>   | <i>grams</i>     |
| I      | 1   | 21.15          | 71.1         | 338.2              | 2590            | 18.66          | + 2.49           |
|        | 2   | 21.15          | 71.8         | 338.2              | 2596            | 20.04          | + 1.11           |
|        | 3   | 21.15          | 71.8         | 338.2              | 2596            | 20.59          | + 0.56           |
|        | 4   | 21.31          | 71.8         | 338.2              | 2600            | 21.31          | ± 0.00           |
| II     | 5   | 21.51          | 221.1        | 000.0              | 2607            | 23.28          | - 1.77           |
|        | 6   | 21.55          | 217.0        | 000.0              | 2570            | 24.03          | - 2.48           |
|        | 7   | 21.55          | 215.5        | 000.0              | 2556            | 26.53          | - 4.98           |
| III    | 8   | 21.10          | 70.4         | 338.2              | 2581            | 21.65          | - 0.55           |
|        | 9   | 21.10          | 70.4         | 338.2              | 2581            | 19.21          | + 1.89           |
|        | 10  | 21.10          | 70.4*        | 338.2              | 2581            | 19.65          | + 1.45           |

Tallquist compared the protein-protecting powers of isodynamic amounts (amounts having equal energy value) of carbohydrates and fats when only a part of either was replaced by the other. The subject was Tallquist himself, a man twenty-eight years old, in good health, and weighing about 80 kilograms. The experiment was performed in Rubner's laboratory, and the diet contained such an amount of total food as was estimated by Rubner to be just about sufficient to supply the energy requirements of the body, viz., 36 Calories per kilogram per day. The experiment covered 8 days divided into two equal periods. In the first four-day period the diet was rich in carbohydrates, in the second period it was rich in fats. There was no change in the nature of the protein fed. All foods furnishing any significant amount of nitrogen were the same in the two periods of the experiment.

The food of the first period consisted of meat, milk, butter, bread, sugar, coffee, beer. That of the second period contained the same amounts of meat, milk, bread, coffee, and beer, but less sugar, more butter, and some bacon. The same amount of salt was taken in each case. The principal data of the experiment are summarized in Table 27.

Here only a part of the carbohydrate, about half of that present and an amount representing about one third of the total fuel value of the diet, was replaced by fat. The change evidently had an unfavorable influence upon the nitrogen balance but the loss of body protein was relatively small and continued only 2 days.

TABLE 27. — NITROGEN BALANCE WHEN FEEDING ISODYNAMIC QUANTITIES OF CARBOHYDRATE AND FAT (Tallquist)

| PERIOD | DAY | INTAKE         |              |                    |              |                 | OUTPUT       | NITROGEN BALANCE |
|--------|-----|----------------|--------------|--------------------|--------------|-----------------|--------------|------------------|
|        |     | Total Nitrogen | Fat          | Carbo-<br>hydrates | Alcohol      | Fuel<br>Value   | Nitrogen     |                  |
|        |     | <i>grams</i>   | <i>grams</i> | <i>grams</i>       | <i>grams</i> | <i>Calories</i> | <i>grams</i> | <i>grams</i>     |
| I      | 1   | 16.27          | 44.0         | 466                | 18.5         | 2867            | 17.11        | — 0.84           |
|        | 2   | 16.27          | 44.0         | 466                | 18.5         | 2867            | 14.40        | + 1.87           |
|        | 3   | 16.27          | 44.0         | 466                | 18.5         | 2867            | 14.65        | + 1.62           |
|        | 4   | 16.27          | 44.0         | 466                | 18.5         | 2867            | 15.58        | + 0.69           |
| II     | 5   | 16.08          | 140.0        | 250                | 19.0         | 2873            | 17.66        | — 1.58           |
|        | 6   | 16.08          | 140.0        | 250                | 19.0         | 2873            | 17.32        | — 1.24           |
|        | 7   | 16.08          | 140.0        | 250                | 19.0         | 2873            | 15.94        | + 0.14           |
|        | 8   | 16.08          | 140.0        | 250                | 19.0         | 2873            | 16.22        | — 0.14           |

Atwater compared the protein-sparing action of carbohydrate and fat in experiments in which the subject, an athletic young man of 76 kilos, performed a considerable amount of work. The experiments were carried out in the respiration calorimeter and covered in all 15 experimental days upon a diet rich in carbohydrates, arranged in four periods which were alternated with four equal periods in which the diet was rich in fats. The change from carbohydrate to fat and vice versa involved about 2000 Calories or nearly half the fuel value of the diet. The average results per day for the entire series of experiments were as shown in Table 28.

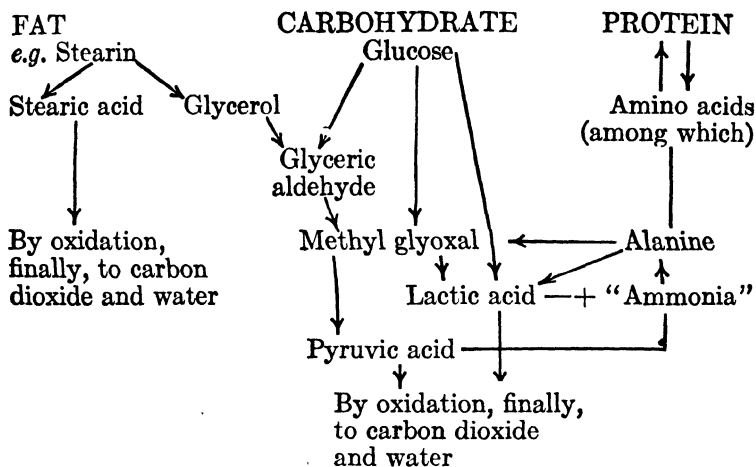
TABLE 28. — NITROGEN BALANCE ON\* DIETS RICH IN CARBOHYDRATES VS. FAT (Atwater)

|   | ON DIET RICH IN<br>CARBOHYDRATE | ON DIET RICH<br>IN FAT |
|---|---------------------------------|------------------------|
| Available Calories in food . . . . .        | 4532                            | 4524                   |
| Heat equivalent of work performed, Calories | 558                             | 554                    |
| Nitrogen in food, grams . . . . .           | 17.5                            | 17.1                   |
| Nitrogen in feces, grams . . . . .          | 2.5                             | 1.7                    |
| Nitrogen in urine, grams . . . . .          | 16.6                            | 18.1                   |
| Nitrogen balance, grams . . . . .           | — 1.6                           | — 2.7                  |

Here again there is a difference in favor of the carbohydrate, but one which is so small as to be of almost no practical significance.

It appears that when the carbohydrate of the food is almost entirely replaced by an equal number of calories in the form of fat there is an unfavorable effect upon the nitrogen balance; but that when the replacement is such as to affect not over one half of the total calories, the difference in protein-sparing action is but slight.

As a *partial explanation* of such experimental facts, the view held by Dakin and many other students of the relationships of the three groups of foodstuffs in the intermediary metabolism may be indicated schematically as follows:



Although ammonium salts do not normally accumulate in the body, it is probable that "ammonia" in some form is constantly being split off in the course of deaminization of the amino acids; and also lactic and/or pyruvic \* acid formed in the intermediary metabolism, especially of carbohydrate. Hence material for the resynthesis of alanine in the body is regularly present, and more abundantly the larger the amount of carbohydrate being transformed. Thus carbohydrate, in undergoing metabolism, presumably spares protein, not only by serving as fuel so that protein need not be drawn upon for this purpose, but also by furnishing material which in combination with ammonia (otherwise a waste product) can actually be converted in the body into at least one of the amino acids of which body proteins are composed and with which they are in equilibrium. This explains how an increased intake of carbohydrate, with resulting increase of available lactic, or pyruvic acid, or both, naturally leads to increased synthesis of

\* The importance of pyruvic acid in the intermediary metabolism has sometimes been questioned but is now strongly supported by the results of research upon the influence of thiamin (vitamin B<sub>1</sub>) upon the pyruvic acid stage of carbohydrate metabolism.

amino acids and thus to a tendency toward protein storage, or, to express the same thing in somewhat different terms, it tends to push the reaction, Amino acids  $\rightleftharpoons$  Protein, toward the right.

According to a widely held view, therefore, most, if not all, of the carbohydrate becomes available through oxidation processes which involve the intermediate production of lactic or pyruvic acid, whose ammonium salts are capable of conversion into amino acid. Of the fat, however, only the glyceryl radicle (about one tenth of the weight, but only about one twentieth of the energy value of the fat) is oxidized through lactic or pyruvic acid, while the fatty acid radicles, representing about nineteen twentieths of the energy of the fat, are metabolized through processes which yield, so far as we know, no product whose ammonium salt is convertible into amino acid in the body. Hence, complete withdrawal of carbohydrate, even though substituted by sufficient fat to yield an equal number of calories, must be expected to result in increased excretion of nitrogen; but when no more than half of the carbohydrate is replaced by fat there seems to be enough lactic and/or pyruvic acid produced to effect economical metabolism of protein.

From what has been said above it will be apparent that, within rather wide limits, the greater the amounts of carbohydrates and fats eaten, the smaller will be the amount of protein metabolized as indicated by the nitrogen output. And, naturally, still lower nitrogen outputs result if there is no attempt at equilibrium and the protein intake is drastically reduced while a liberal intake of total calories is maintained.

The concept above outlined also goes far to explain the fact (rediscovered and perhaps somewhat overemphasized every decade or two) that protein can in some degree be spared by feeding its partially reversible metabolite such as suitable ammonium salts, or even urea, each of which tends to reduce the rate of protein expenditure in the body by checking the reaction of which it is a product, according to the general principles of dynamic equilibria and concentration ("mass action") effects. Another part of the explanation is the possible synthesis of proteins from simpler nitrogen compounds by the action of microorganisms in the digestive tract. Moreover, both the above processes of protein "sparing" or "replacing" by simpler nitrogen compounds may be active at the same time.

### Nitrogen Output When Diet Is Nearly Protein-free

The figures in Table 29 are typical of the data compiled by Sotola (1928) from experiments upon different species with diets of about adequate energy value and almost negligible nitrogen (protein) content.

TABLE 29. — LOW OUTPUTS OF NITROGEN BY DIFFERENT SPECIES (Sotola)

| SUBJECT      | BODY WEIGHT      | NITROGEN OF URINE<br>PER KILOGRAM<br>BODY WEIGHT | INVESTIGATOR       |
|--------------|------------------|--|--------------------|
|              | <i>kilograms</i> | <i>grams</i>                                     |                    |
| Man . . .    | 85.7             | 0.042  | Folin              |
| Man . . .    | 72.4             | 0.047  | Graham and Poulton |
| Man . . .    | 60.5             | 0.035  | Robison            |
| Pig . . .    | 68.4             | 0.039  | McCollum           |
| Lamb . . .   | 24.3             | 0.033  | Sotola             |
| Dog . . .    | 11.3             | 0.158  | Murlin             |
| Rabbit . . . | 1.74             | 0.126  | Mendel and Rose    |
| Rat . . .    | 0.134            | 0.220  | Mitchell           |

Deuel (1928) lived continuously for 54 days upon a diet nearly free from protein, consisting of starch, sugar, orange juice clarified by centrifugation, lettuce, cod liver oil, and definite amounts of pure salts. This diet contained usually from 0.24 to 0.32 gram (and never more than 0.31 gram) of total nitrogen per day, and not all of this was in the form of protein. The energy value was about 1800 Calories per day, chiefly derived from carbohydrate. On this diet the urinary output of nitrogen fell rapidly from nearly 10 grams on the first day to less than 4 grams per day at the end of a week, and about 2 grams per day at the end of a month. Throughout the month, however, the output of creatinine nitrogen remained nearly constant, beginning at 0.60 gram and ending at 0.56 gram per day. The data of Table 30 indicate the rates of (urinary) output of the chief forms of nitrogen as the experiment progressed.

At the end of a month on this nearly protein-free diet and when the total urinary nitrogen output had become nearly constant at about 2 grams per day, thyroxine was administered and resulted in a temporary increase of the nitrogen excretion to a maximum of 6.12 grams on the 8th day of this thyroxine period. Thereafter this nitrogen output decreased in the course of four days to about 3 grams per day and remained at this low rate notwithstanding the administration of sufficient thyroxine to maintain the increased rate of energy metabolism. The increased nitrogen excreted under the influence of thyroxine appeared almost wholly as urea, creatinine excretion remaining essentially constant. This result was taken as confirming the view that even under these conditions the extra protein metabolized was "dispensable" rather than "body tissue" protein.

In discussing these results, Lusk remarked that, "The protein reserves of the body are relatively enormous," and estimated that, with carbohydrate to supply the needed energy, a man starting from an ordinary condition of nutrition could probably live for more than a year without any protein whatever in his food (*Science of Nutrition*, 4th Ed., p. 361).

Without attempting to discuss the vexed question of possible injury from abnormally high or low intakes of protein, let us now study the data which bear directly upon the problem of the normal protein requirement.

TABLE 30. — DAILY OUTPUT OF NITROGEN IN DIFFERENT FORMS (Deuel)

|              | TOTAL<br>NITROGEN | UREA<br>NITROGEN | AMMONIA<br>NITROGEN | CREATININE<br>NITROGEN | URIC-ACID<br>NITROGEN |
|--------------|-------------------|------------------|---------------------|------------------------|-----------------------|
|              | <i>grams</i>      | <i>grams</i>     | <i>grams</i>        | <i>grams</i>           | <i>grams</i>          |
| 1st day . .  | 9.73              | 7.42             | 0.33                | 0.60                   | 0.15                  |
| 2nd day . .  | 8.18              | 5.85             | 0.29                | 0.59                   | 0.18                  |
| 3rd day . .  | 7.35              | 4.99             | 0.22                | 0.57                   | 0.20                  |
| 4th day . .  | 6.05              | 3.88             | 0.20                | 0.57                   | 0.19                  |
| 5th day . .  | 5.49              | 3.49             | 0.25                | 0.57                   | 0.18                  |
| 6th day . .  | 4.82              | 2.76             | 0.21                | 0.57                   | 0.15                  |
| 7th day . .  | 4.42              | 2.45             | 0.24                | 0.57                   | 0.17                  |
| 8-9 days . . | 3.52              | 2.02             | 0.18                | 0.57                   | 0.16                  |
| 10-16 days . | 3.29              | 1.87             | 0.17                | 0.56                   | 0.16                  |
| 17-23 days . | 2.91              | 1.63             | 0.15                | 0.56                   | 0.16                  |
| 24-29 days   | 2.32              | 1.24             | 0.10                | 0.56                   | 0.14                  |
| 30th day .   | 2.10              | 1.14             | 0.10                | 0.56                   | 0.13                  |

### Protein Requirement of Adult Maintenance

The practical problem may be stated thus: When the total food is properly adjusted to the size and activity of the subject so that there is sufficient but not excessive fuel to meet all the energy requirements, how much protein must the daily food contain in order to keep the body in nitrogen equilibrium?

Chittenden based his estimate of the protein requirement, not only upon the nitrogen balances, but also upon the amounts of nitrogen observed to be eliminated daily through the kidneys over long periods in which the body may or may not have been in equilibrium, but in which health and efficiency were certainly maintained. The first men to serve as subjects in this investigation were Chittenden himself and his associates, all of whom continued their professional work and either reported no effect or felt benefited by the change to the lower-protein diet. Similar experiments were then made upon a squad of soldiers, who were quartered near the laboratory during the test and given regular exercise in the gymnasium in addition to light duties about their quarters. These men showed marked improvement in physical condition during the test, probably due in part to their more regular habits of life and their gymnastic exercises. In order to eliminate this latter factor while still applying the low-protein diet to young and physically active men, the investigation was extended to cover a group of university athletes who were already well trained and in prime physical condition at the beginning of their



dietary experiment. As Chittenden emphasized, these athletes not only maintained, but in many cases improved, their gymnastic records while on the low-protein diet, one of them winning an all-round gymnastic championship during the time. Chittenden states \* that his data "are seemingly harmonious in indicating that the physiological needs of the body are fully met by a metabolism of protein matter equal to an exchange of 0.10 to 0.12 gram of nitrogen per kilogram of body weight per day, provided a sufficient amount of non-nitrogenous foods is taken to meet the energy requirements of the body." This would correspond to 44 to 53 grams of protein per day for a man of average weight (70 kilograms, 154 pounds, without clothing).

In an examination made in 1920 of the available literature upon this subject there were found 109 experiments upon adults showing no abnormality of digestion or health, in which the diet was sufficiently well adjusted to the probable requirement and the nitrogen balance showed sufficient approach to equilibrium to make it appear that the total output of nitrogen might be taken as an indication of the protein requirement. These experiments were made in the course of 25 independent investigations in which 47 different individuals (39 men and 8 women) served as subjects. For purposes of comparison the daily output of total nitrogen in each experiment was calculated to protein and this to a basis of 70 kilograms of body weight. Reckoned in this way, the apparent protein requirement as indicated by the data of individual experiments ranged between the extremes of 21 and 65 grams, averaging 44.4 grams of protein per 70 kilograms of body weight per day.

Average results for men and for women were practically identical when calculated to the same basis of body weight (for women 44.6 grams, and for men 44.3 grams, per 70 kilograms).

Study of the data recorded in the original papers indicates that the differences in amounts of protein catabolized in the different experiments cannot be attributed primarily to the kind of protein consumed nor to the use of diets of fuel values widely different from the energy requirements. Apparently, the most influential factor was the extent to which the subject had become accustomed to a relatively low protein intake such as is best calculated to throw light upon the question of the actual requirement. In the desire to avoid any danger of arbitrary selection of data,

\* *Nutrition of Man*, pages 226, 272.

the writer probably erred in the direction of including some experiments which gave misleadingly high results because of too short periods on the low-protein diets. The *best* data would probably yield an average result not far from 0.5 gram protein per kilogram of body weight per day for normal adult maintenance after allowing a reasonable period for adjustment to such a low-protein diet.

### **Difference between Actual Requirement and "Standard" Allowance of Protein**

It may be well to point out here the distinction between the amount of protein actually required, on the one hand, and, on the other hand, the amount which it may be thought best to allow in the planning of dietaries. The term *requirement* is here applied only to the former; the latter may better be called the *protein allowance* or the "standard" for protein. The difference between the amount actually required and the amount which would ordinarily be allowed in planning a dietary is much greater with protein than with fuel value. Surplus fuel is stored as fat, and if excessive fatness is to be avoided, the fuel value of the food must not greatly exceed the energy requirements of the body; but surplus nitrogen (or most of it) is rapidly eliminated from the body and, so long as no injury to health results, leaves no evidence of having been taken in excess of body needs. The eating of a considerable surplus of protein is habitual, and such a surplus of protein in the food is believed by many people to constitute a desirable "factor of safety," if not indeed to exert a directly beneficial effect upon health and stamina.

### **Effects of Different Levels of Protein Intake upon Normal Nutrition throughout the Life Cycle**

For obvious reasons it is not feasible to extend controlled experiments upon human beings throughout the life cycle. This, however, can be done with suitably selected experimental animals; and among these the laboratory rat is especially suitable. Among other advantages, the rat is much like man in its omnivorous food habits and in the fact that the chemistry of its nutrition is strikingly like that of the human being. For these and other reasons explained in their publications, Osborne and Mendel made much use of rats in studying the values of individual proteins for the purposes of human nutrition, as briefly indicated in Chapter V.

Slonaker (1931, 1935, 1938) fed rats throughout their natural lives on five different diets containing approximately 10, 14, 18, 22, and 26 per cent, respectively, of protein calories in the total food calories. Lean meat constituted the source of the extra protein. The general outcome indicated that the best permanent results were obtained with protein at the 14 per cent level. Analogous full-life experiments in which the extra protein was given in the form of casein (work of Ellis, of Conner and Kao, and of Kao and Conner in the Columbia laboratories) yielded results in general agreement with Slonaker's though somewhat more uniformly favorable throughout the range of 14 to 25 per cent of protein in the dry food or protein calories in the total food calories. These results undoubtedly have significance for the human problem; though it is still an open question whether the quantitative level should be regarded as carrying over directly, or whether in view of the much slower rate at which human beings run through their life cycles and the relatively lesser demands of pregnancy and lactation in human life, we should interpret the results as indicating that the optimal average protein intake for life in the case of the human being should be somewhat less than 14 per cent of the total calories. At any rate the results of recent research confirm and strengthen the generally accepted dietetic custom of allowing from 10 to 15 per cent of the total food calories for protein under ordinary conditions.

**Influence of the Choice of Food upon Protein Requirement:  
Nutritional Efficiency of the Protein-Mixtures Contained  
in Different Foods**

When isolated proteins are fed singly, striking differences in nutritive value appear, as has been shown in Chapter V. In view of this fact it may seem strange that in the experiments hitherto conducted to determine the protein requirement of man the kind of protein fed has not exerted a more striking influence upon the results obtained. There is, however, no real discrepancy between the two sets of findings. The experiments made for the purpose of comparing individual proteins were conducted largely upon rapidly growing young animals, in which there is an active synthesis and retention of protein, so that a deficiency in the supply of any amino acid which is required in the construction of body protein is apt to be quickly and plainly reflected in a diminution or ces-

sation of growth. On the other hand, in experiments like those described in the preceding section, where the purpose is not to compare proteins but to measure the normal protein requirement, the diet is naturally made up, not of isolated proteins or even of single or unusual foods, but (ordinarily at least) of such combinations of staple foods as are believed to represent a normal diet, so that even a relatively simple ration arranged for the purposes of such an experiment would probably contain a number of different proteins among which any peculiarities of amino-acid constitution would be more or less apt to offset each other. Moreover, the experiments of the latter group (by definition) have been made entirely upon adults whose protein requirement was limited to that of maintenance. The amount of an essential amino acid required for the so-called repair processes of maintenance may be relatively less than is needed in the construction of wholly new tissue in growth. Osborne and Mendel have suggested that in the protein metabolism of maintenance the need for a particular amino acid may be not so much for repair of tissue as to serve as the precursor of some essential hormone. It may also be helpful to think of the protein metabolism not only in terms of building and repair, but also of maintaining the (approximate) dynamic equilibrium which exists between proteins and amino acids in the cells of the animal tissues.

That there is always an exchange between amino-acid radicles of the proteins of living tissue and the free amino acids of the same kind in the tissue fluid was made sufficiently clear by the independent work of Dakin and of McCollum nearly three decades ago, and has been recognized in previous editions of this book. It now becomes easier to demonstrate by experiments with amino acids which are "tagged" by containing a readily detectable isotopic atom. Thus, Ratner, Rittenberg, Keston, and Schoenheimer (1940) feeding glycine containing heavy nitrogen to full-grown well nourished rats found that much of the free glycine thus fed replaced glycine of body protein. Over one per cent of the glycine of the entire body protein (and a much higher proportion of that of the liver) was "heavy-nitrogen glycine" three days after the feeding. Some of the heavy nitrogen had also transferred from glycine radicles to the radicles of other amino acids.

Similarly the concept of a series of dynamic near-equilibria

explains the "sparing" of protein by the feeding of such metabolites as ammonium carbonate or even urea.

Concentration of any of the amino acids into which tissue proteins tend to be hydrolyzed may be expected to help in pushing the reaction, Amino acids  $\rightleftharpoons$  Protein, toward the right; in other words, *any* of these amino acids may thus function in the *maintenance* of body protein, whereas for the synthesis of new protein as in *growth*, *all* the amino acids which enter into the structure of tissue proteins would be needed. Hence it is quite reasonable that proteins of different efficiency for growth may show much more nearly equal efficiency in the normal maintenance nutrition of adults; though it is also true that, so far as known, the proteins more efficient for growth are likewise more efficient for maintenance.

Numerous and carefully conducted experiments have now shown the possibility of healthy adult maintenance with nitrogen equilibrium upon dietaries furnishing not over 0.5 gram of protein per kilogram of body weight per day, even when the food protein was not of the highest nutritional efficiency as shown by growth experiments.

This was true, for example, in the experiments of Rose and Cooper with potato and of Sherman with bread as the sources of protein, in both of which nitrogen equilibrium was maintained on 0.5 gram of protein per kilogram of body weight per day; and in those of Sherman and Winters in which a young woman showed similar results upon a diet in which about nine tenths of the protein was from corn (maize) meal and about one tenth from milk.

Rose, MacLeod, and Bisbey experimented with diets in which the protein was furnished: (1) almost entirely by meat; (2) almost entirely by milk; (3) almost entirely by a mixture of bread and milk in such proportions that the protein came in practically equal amounts from those two foods. The food furnished 0.08 gram of nitrogen per kilogram of body weight per day. Nitrogen was stored in the body in all three cases as follows: (1) 0.06 gram per day; (2) 0.55 gram; (3) 0.41 gram. Here the protein of milk and that of the bread-and-milk mixture proved measurably more efficient than the protein of meat.

Hart and Steenbock secured satisfactory growth in young swine when about one third of the total protein of the food was furnished by milk and about two thirds by maize, whereas if milk alone

or grain alone were the sole source of protein, the proteins of milk showed at least twice the nutritive efficiency of the grain proteins.

As in growth, so in lactation, the demand for material for the construction of new protein creates a condition in which differences of value in the protein fed may readily become more apparent than when only maintenance is involved. Hart and Humphrey found that in meeting the protein requirements of milch cows, milk protein and the protein of linseed meal are about 50 per cent more efficient than the proteins of the corn (maize) or of the wheat kernel; and Hoobler has shown that milk is an exceptionally good form of food protein for the production of human milk and the protection of the body protein of the nursing mother.

Methods of studying the relative "biological values" of the proteins of different foods in the special and rather artificial sense in which this term has come to be used, do not fall within the scope of this book.

### **Influence of Muscular Exercise**

At one time it was supposed that muscular power was generated at the expense of muscle substance, and this necessitated the belief that muscular work always increased protein metabolism. Since we now know that the muscles work quite as well at the expense of carbohydrates and fats as of protein, the conclusion that muscular work necessarily increases the metabolism of protein is far from inevitable. It is only necessary to observe the effects of regular muscular exercise, either in athletic training or in manual labor, to see that normally the muscles do not waste away when thus used, but rather tend to become larger. Such a growth of the muscles tends toward a storage rather than a loss of protein. Usually, however, muscular work also results in increased appetite, and it is difficult to separate the effects of the exercise from those of the extra food.

Whether muscular work acts directly to increase the amount of protein metabolized in the body can only be determined by experiments in which sufficient extra fats and carbohydrates are fed to furnish the extra fuel required on the working days. But since fats and carbohydrates spare protein, the feeding of these in any excess over just what is necessary to provide for the increased energy requirement would tend to decrease the metabolism of protein and counteract any effect which the muscular

work might otherwise have in increasing protein metabolism. Hence, in order to show conclusively whether muscular work of itself has any influence upon the protein metabolism, it would be necessary to determine the mechanical efficiency of the man, then to bring him into equilibrium with an amount of food just sufficient for his needs, and finally to have him perform a measured amount of work, at the same time adding to his diet an amount of fats and carbohydrates just sufficient to furnish the extra energy required for the work performed. Such elaborate experiments have not yet been made, but we have sufficient data to show that they are not necessary for practical purposes. Many experiments have shown conclusively that increased work, when accompanied by a sufficient increase in the amount of fats and carbohydrates fed, does not necessarily increase the metabolism of protein.

Table 31 gives data from Atwater (*Report of the Storrs, Connecticut, Agricultural Experiment Station for 1902-1903*, page 127) which show the average results of a long series of rest and work experiments with men in the respiration calorimeter.

TABLE 31. — MUSCULAR WORK AND PROTEIN METABOLISM (Atwater)

| NATURE OF EXPERIMENT  | AVERAGE METABOLISM PER DAY |              |                          |              |                          |              |
|---|----------------------------|--------------|--------------------------|--------------|--------------------------|--------------|
|   | Per Person                 |              | Per Kilogram Body Weight |              | Per Square Meter Surface |              |
|   | Energy                     | Protein      | Energy                   | Protein      | Energy                   | Protein      |
|   | <i>Calories</i>            | <i>grams</i> | <i>Calories</i>          | <i>grams</i> | <i>Calories</i>          | <i>grams</i> |
| <i>Rest:</i> Food generally sufficient for equilibrium; 5 subjects, 27 experiments, covering 82 days.                                     | 2310                       | 103.8        | 33.5                     | 1.51         | 1116                     | 50.1         |
| <i>Work:</i> 8 hours per day. Food generally not quite sufficient for equilibrium; 3 subjects, 24 experiments, covering 76 days . . . . . | 4556                       | 108.1        | 62.9                     | 1.49         | 2129                     | 50.5         |

Comparing the figures either per unit of weight or of surface, it will be seen that muscular work sufficient nearly to double the energy metabolism had no appreciable effect upon the amount of protein metabolized as measured by the nitrogen output.

Considering the large amount of exceptionally accurate research represented in these figures, they seem to justify the conclusion that if muscular work has any tendency to increase the metabolism of muscle substance, such effect is normally balanced by the tendency of the muscles to grow (and therefore to store protein) when exercised.

Moreover, it is certain that any effect which muscular work might possibly have in increasing protein metabolism would be incomparably less than its effect in increasing the energy metabolism. If, then, starting with a diet which maintains protein equilibrium at rest, the total food is increased sufficiently to provide for the muscular work, and the increase in the diet is accomplished by adding any reasonable combination of food materials, we may feel sure that these will supply plenty of protein to meet any possible increase in the protein requirement. Hence, in planning the diet of a man at hard muscular work, any reasonable combination of foodstuffs given in sufficient abundance to meet the energy requirement will almost certainly supply an ample amount of protein.

### Protein Requirement in Relation to Age and Growth

If a man at moderately active work takes a diet which furnishes 3000 Calories and 75 grams of protein, he is taking 10 per cent of his food-energy in the form of protein. Let this be compared with the normal dietary of an infant. Human milk averages about 1.6 per cent protein, 4.0 per cent fat, 7.0 per cent carbohydrate. Here about 9 per cent of the calories are taken in the form of protein, or about the same proportion as has been allowed for the full-grown active man. Furthermore, Hoobler has shown experimentally that this is as high a proportion of protein as the infant is likely to utilize with the best efficiency in growth of body tissue. During the suckling period the growth is relatively more rapid than at any other age. Mendel \* gives the following figures:

#### THE RELATIVE DAILY GAIN IN BODY WEIGHT OF CHILDREN

|                                 |               |
|---------------------------------|---------------|
| In the first month is about     | 1.00 per cent |
| At the middle of the first year | 0.30 per cent |
| At the end of the first year    | 0.15 per cent |
| At the fifth year               | 0.03 per cent |
| Maximum in later years          |               |
| for boys                        | 0.07 per cent |
| for girls                       | 0.04 per cent |

\* *Childhood and Growth*, page 18.



If, then, the full-grown man and the child at the time of most rapid growth each requires but 10 per cent of his calories in the form of protein, it seems probable that this proportion is also sufficient for any intermediate age, if the diet is of ample fuel value, and the protein is of the right kind. But the proper selection of the protein is important in the feeding of children, who differ from most other young mammals in that their period of growth is so many times longer than the suckling period. Even the child that is nursed for a year and attains three times his birth weight before weaning will still have much the greater part (probably five sixths) of his growth to make on other food than his own mother's milk. By the time growth is complete he will probably have about twenty times the body weight and possibly more than twenty times the body protein with which he was born.

Growth at the normal rapid rate of early childhood involves the conversion of a very considerable part, sometimes as much as one third, of the protein of the food into body protein. This is accomplished to the best advantage when: (1) the protein of the food is largely of the kind most efficient in supporting growth, *i.e.*, milk protein; (2) the protein is well protected by the protein-sparing action of liberal amounts of carbohydrate and fat.

That the child needs a diet of high fuel value to meet the requirements of his energy metabolism has already been pointed out (Chapters IX and X). It is because the high protein requirement of childhood (for young children more than twice as much per unit of weight as for adults) is paralleled by an equally high energy requirement that the diet of the child need not contain a higher percentage of its calories in the form of protein than does the ordinary diet of the adult, if the protein for the child is well chosen.

Usually, however, a well-planned dietary for a child will show a somewhat more than average proportion of its calories in the form of protein because after weaning the main feature of the child's diet should be cow's milk, which furnishes about 19 per cent of its calories in the form of protein. A child fed mainly upon cow's milk and taking enough food amply to cover his energy requirement will therefore receive a safe surplus of protein in highly efficient form. With an optimal amount of milk in the dietary of the growing child, the other foods may be selected with primary reference to other qualities than their protein content; without a liberal use of milk the proper feeding of a growing child

becomes a very difficult problem, even more with respect to mineral and vitamin than to protein needs.

Having discussed the protein needs of ordinary adult maintenance and of growth, the needs of the aged should also be considered. This does not require extended discussion, since advancing age involves no new features but only a gradual modification of those pertaining to middle life.

In general, elderly people show a somewhat diminished protein requirement and likewise a diminished power of dealing with excess. Surplus protein taken in the food is not so rapidly absorbed and catabolized, and, while there appears to be no essential difference in the form in which the nitrogen is finally excreted, the susceptibility to excessive putrefaction of protein appears to be increased. It would seem that in the dietary of the aged the protein should be reduced to at least as great an extent as are the calories..

### Possibility of Protein Deficiency Conditions Not Attributable to Fault of the Food

Sometimes a pathological instead of physiological functioning of the body may result in a protein-poor condition of the blood serum, although the food supplies ample protein to meet all normal needs. The papers by Weech and by Weech and Goettsch listed below deal in part with such a condition. Although dietary measures may alleviate such conditions, nevertheless the problem is a medical one and therefore lies beyond the scope of this book. From the standpoint of the practice of medicine, the recognition of such conditions may give rise to the view that protein is deserving of renewed attention; but this does not call for any increase in our estimate of the protein need of *normal* nutrition. Rather it calls for *discrimination between* the responsibilities of everyday food planning for normal nutrition, *and* the management of a medical problem in deranged metabolism.

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## CHAPTER XII

### MINERAL ELEMENTS IN FOODS AND NUTRITION

#### The Elementary Composition of the Body

FROM various estimates by different writers, the average elementary composition of the human body may be presumed to be approximately as shown in the first half (at the reader's left) of the accompanying table. The other half of the same table shows Clarke's estimate of the elementary composition of the environment in and from which we have evolved: the earth's crust with its oceans and atmosphere. Clarke (1920) based his estimate on elaborate studies of the composition of as much of the earth's solid crust as is within ten miles of sea-level, together with the oceans and other waters of the earth's surface and the air of its weighable atmosphere. The elements which Clarke estimated as constituting less than 0.03 per cent of this total are here omitted for the sake of brevity. Of the elements reported as found in the body, we cannot always be sure which are essential to its structure or functioning and which are of accidental occurrence. All of those for which figures are given in Table 32, and also cobalt, are now definitely regarded as essential; while opinion still differs as to whether zinc and a number of other elements are nutritionally essential.

The prominence of *oxygen* in the elementary composition of our bodies is, of course, largely due to the fact that about two thirds of the body weight is water; but also oxygen is a constituent of nearly all of the compounds in the body.

Its prominence as a constituent both of water and of the organic body-stuffs gives *hydrogen* a high place in the quantitatively arranged list of the body's essential elements, and accounts for its constituting a higher percentage of the body than of its environment.

Of *carbon* and also of *nitrogen* the body contains about 100-fold higher concentration than does its environment. This is chiefly due to the fact that the body tissues are so largely composed of carbon and nitrogen compounds.



Compounds of carbon, nitrogen, hydrogen, and oxygen have been studied in part both as foodstuffs and body constituents in the preceding chapters, and will also be studied further in the chapters on vitamins.

Such other chemical elements as are normally essential to the structure or function of the body are commonly called the mineral elements of foods and nutrition, or the inorganic foodstuffs, or the ash constituents; and their transformations and

TABLE 32. — ELEMENTARY COMPOSITIONS OF THE BODY AND ITS ENVIRONMENT COMPARED

| APPROXIMATE ELEMENTARY COMPOSITION<br>OF THE ADULT HUMAN BODY<br>(Data from many sources) |                  | APPROXIMATE ELEMENTARY COMPOSITION<br>OF THE EARTH'S CRUST WITH ITS OCEANS<br>AND ATMOSPHERE (Clarke) |            |
|---|------------------|---|------------|
| Chemical Element  | Percentage       | Chemical Element  | Percentage |
| Oxygen . . . . .  | 65.              | Oxygen . . . . .  | 50.02      |
| Carbon . . . . .  | 18.              | Silicon . . . . .   | 25.80      |
| Hydrogen . . . . .  | 10.              | Aluminum . . . . .  | 7.30       |
| Nitrogen . . . . .  | 3.0              | Iron . . . . .  | 4.18       |
| Calcium . . . . .   | 2.2 <sup>a</sup> | Calcium . . . . .   | 3.22       |
| Phosphorus . . . . .  | 1.2 <sup>b</sup> | Sodium . . . . .  | 2.36       |
| Potassium . . . . .   | 0.35             | Potassium . . . . .   | 2.28       |
| Sulfur . . . . .  | 0.25             | Magnesium . . . . .   | 2.08       |
| Sodium . . . . .  | 0.15             | Hydrogen . . . . .  | 0.95       |
| Chlorine . . . . .  | 0.15             | Titanium . . . . .  | 0.43       |
| Magnesium . . . . .   | 0.05             | Chlorine . . . . .  | 0.20       |
| Iron . . . . .  | 0.004            | Carbon . . . . .  | 0.18       |
| Manganese . . . . .   | 0.0003           | Phosphorus . . . . .  | 0.11       |
| Copper . . . . .  | 0.00015          | Sulfur . . . . .  | 0.11       |
| Iodine . . . . .  | 0.00004          | Fluorine . . . . .  | 0.10       |
| Cobalt . . . . .  | ( <sup>c</sup> ) | Barium . . . . .  | 0.08       |
| Zinc . . . . .  | ( <sup>c</sup> ) | Manganese . . . . .   | 0.08       |
| Others found, but of doubtful function  |                  | Nitrogen . . . . .  | 0.03       |
|   |                  | Others in lesser amounts  |            |

<sup>a</sup> Estimates of normal calcium content vary widely.

<sup>b</sup> Phosphorus varies with calcium.

<sup>c</sup> Believed to be essential, but as yet no consensus of opinion upon quantitative estimates.

“exchange” in the body collectively may be called the mineral metabolism. None of these terms is entirely satisfactory. Some of the elements which remain in the ash when a food is burned may have existed largely as constituents of organic compounds in the food itself. With the facts clearly understood, any of the current designations for these elements may be used.

### General Functions of Mineral Elements in Nutrition

The elements concerned in the mineral metabolism may exist in the body and take part in its functions in at least three kinds of ways:

(1) As constituents of the bones and teeth, giving rigidity and relative permanence to the skeletal tissues;

(2) As essential elements of the organic compounds which are the chief solid constituents of the soft tissues (muscles, blood cells, etc.);

(3) As soluble salts (electrolytes) held in solution in the fluids of the body, giving these fluids their characteristic influence upon the elasticity and irritability of muscle and nerve, supplying the material for the acidity or alkalinity of the digestive juices and other secretions, and yet maintaining the approximate neutrality of the body's fluids as well as their osmotic pressure and solvent power.

A man under average conditions of diet, activity, and health usually excretes daily from 20 to 30 grams of mineral salts, consisting mainly of chlorides, sulfates, and phosphates of sodium, potassium, magnesium, and calcium (as well as variable small amounts of ammonium salts from the protein metabolism).

In a fast of 31 days recorded by Benedict (1915) the elimination of several elements was determined quantitatively from day to day or in periods of two to three days with the results shown in Table 33.

It will be noted that each of the elements seems to run its own course except that (1) the sulfur tends to remain in a more or less parallel relation to the nitrogen (both being involved in protein metabolism); and (2) the output of sodium tends to run parallel with that of chlorine, since these two elements are excreted mainly as common salt.

In order to support permanently normal nutrition, the intake of each essential element must of course be sufficient to cover the output; and, in the case of the growing body, to provide an additional amount for daily storage as a constituent of the newly formed body substances. Instead of the former assumption, that in the practical feeding of people and of farm animals all this can safely be left to chance, there is now very great interest in mineral metabolism on both theoretical and practical grounds. Not only

TABLE 33. — URINARY EXCRETION OF DIFFERENT ELEMENTS DURING A 31-DAY FAST (Benedict)

| DAY | NITRO-<br>GEN | CHLO-<br>RINE | PHOS-<br>PHORUS | SULFUR       | CALCIUM      | MAGNE-<br>SIUM | POTAS-<br>SIUM | SODIUM       |
|-----|---------------|---------------|-----------------|--------------|--------------|----------------|----------------|--------------|
|     | <i>grams</i>  | <i>grams</i>  | <i>grams</i>    | <i>grams</i> | <i>grams</i> | <i>grams</i>   | <i>grams</i>   | <i>grams</i> |
| 1   | 7.10          | 3.77          | 0.73            | 0.46         | 0.217        | 0.046          | 1.630          | 2.070        |
| 2   | 8.40          | 1.02          | 1.08            | 0.61         | .243         | .106           | 1.368          | .926         |
| 3   | 11.34         | 0.79          | 1.10            | 0.68         | .243         | .106           | 1.368          | .926         |
| 4   | 11.87         | 0.59          | 1.27            | 0.67         | .243         | .106           | 1.368          | .926         |
| 5   | 10.41         | 0.41          | 1.15            | 0.65         | .274         | .098           | 1.445          | .276         |
| 6   | 10.18         | 0.40          | 1.02            | 0.65         | .274         | .098           | 1.445          | .276         |
| 7   | 9.79          | 0.55          | 0.80            | 0.62         | .253         | .070           | .883           | .154         |
| 8   | 10.27         | 0.32          | 0.80            | 0.64         | .253         | .070           | .883           | .154         |
| 9   | 10.74         | 0.31          | 0.93            | 0.66         | .253         | .070           | .883           | .154         |
| 10  | 10.05         | 0.28          | 0.86            | 0.61         | .220         | .072           | 1.006          | .100         |
| 11  | 10.25         | 0.36          | 0.85            | 0.62         | .220         | .072           | 1.006          | .100         |
| 12  | 10.13         | 0.31          | 0.74            | 0.62         | .216         | .065           | —              | —            |
| 13  | 10.35         | 0.32          | 0.85            | 0.62         | .216         | .065           | —              | —            |
| 14  | 10.43         | 0.26          | 0.81            | 0.60         | .236         | .071           | .814           | .109         |
| 15  | 8.46          | 0.16          | 0.64            | 0.50         | .236         | .071           | .814           | .109         |
| 16  | 9.58          | 0.14          | 0.89            | 0.59         | .214         | .078           | —              | —            |
| 17  | 8.81          | 0.12          | 0.87            | 0.53         | .214         | .078           | —              | —            |
| 18  | 8.27          | 0.15          | 0.81            | 0.54         | .251         | .059           | .676           | .051         |
| 19  | 8.37          | 0.16          | 0.77            | 0.55         | .251         | .059           | .676           | .051         |
| 20  | 7.69          | 0.15          | 0.64            | 0.51         | .237         | .053           | .644           | .066         |
| 21  | 7.93          | 0.18          | 0.70            | 0.51         | .237         | .053           | .644           | .066         |
| 22  | 7.75          | 0.21          | 0.69            | 0.50         | .179         | .050           | .643           | .083         |
| 23  | 7.31          | 0.18          | 0.71            | 0.51         | .179         | .050           | .643           | .083         |
| 24  | 8.15          | 0.10          | 0.68            | 0.49         | .167         | .056           | .787           | .065         |
| 25  | 7.81          | 0.18          | 0.67            | 0.49         | .167         | .056           | .787           | .065         |
| 26  | 7.88          | 0.16          | 0.65            | 0.54         | .153         | .051           | .656           | .055         |
| 27  | 8.07          | 0.16          | 0.62            | 0.52         | .153         | .051           | .656           | .055         |
| 28  | 7.62          | 0.14          | 0.59            | 0.53         | .131         | .047           | .585           | .036         |
| 29  | 7.54          | 0.12          | 0.64            | 0.52         | .131         | .047           | .585           | .036         |
| 30  | 7.83          | 0.14          | 0.61            | 0.52         | .138         | .052           | .606           | .053         |
| 31  | 6.94          | 0.13          | 0.58            | 0.49         | .138         | .052           | .606           | .053         |

do the different food materials differ widely in the absolute and relative abundance of the different elements, but the same is also true of the total food intake of different groups of people. Studies of 150 freely chosen American dietaries, each covering the food of a group of people for a week or more, revealed the range shown in Table 34.

It will be seen that the intake of any given element may be widely different in the different dietaries, even though each repre-

TABLE 34. — MINERAL ELEMENTS IN 150 AMERICAN DIETARIES

| ELEMENTS                | PER MAN PER DAY |              |              | PER 3000 CALORIES |              |              |
|-------------------------|-----------------|--------------|--------------|-------------------|--------------|--------------|
|                         | Minimum         | Maximum      | Average      | Minimum           | Maximum      | Average      |
|                         | <i>grams</i>    | <i>grams</i> | <i>grams</i> | <i>grams</i>      | <i>grams</i> | <i>grams</i> |
| Calcium . .             | 0.24            | 1.87         | 0.73         | 0.35              | 1.47         | 0.73         |
| Magnesium .             | 0.14            | 0.67         | 0.34         | 0.17              | 0.53         | 0.34         |
| Potassium .             | 1.43            | 6.54         | 3.39         | 1.63              | 5.27         | 3.40         |
| Sodium <sup>a</sup> .   | 0.19            | 4.61         | 1.94         | 0.22              | 4.83         | 1.95         |
| Phosphorus .            | 0.60            | 2.79         | 1.58         | 0.72              | 2.30         | 1.59         |
| Chlorine <sup>a</sup> . | 0.88            | 5.83         | 2.83         | 0.83              | 7.26         | 2.88         |
| Sulfur . .              | 0.51            | 2.82         | 1.28         | 0.80              | 2.35         | 1.30         |
| Iron . .                | 0.0080          | 0.0307       | 0.0173       | 0.0090            | 0.0234       | 0.0174       |

<sup>a</sup> Since these dietary records did not show the quantities of table salt used, the figures for sodium and chlorine in the table cover only the amounts in the food as purchased and are very greatly below the actual intake of these elements.

sents the daily average for at least a week. To some extent this is due to the variable amounts of total food consumed, but even when the data are reduced to a uniform basis of 3000 Calories the differences are still large. When we find, as here, that a day's food, even averaged over a period of a week or more, may contain anywhere from 0.24 to 1.87 grams of calcium; 0.14 to 0.67 gram of magnesium; 1.43 to 6.54 grams of potassium, it is evident that not all of these can have been optimal intakes, and that the mineral elements of food and their functions in nutrition are subjects calling for careful quantitative study.

The purpose of this chapter is to sketch the mineral metabolism in as concise and connected a manner as practicable, after which the more detailed and quantitative study of the four elements (calcium, phosphorus, iron, and iodine) which assume special prominence in the practical problems of nutrition will be taken up in the chapters which follow.

### Metabolism of Chlorides — Use of Common Salt

Practically all the chlorine involved in metabolism enters, exists in, and leaves the body in the form of chloride ions. The amount of sodium chloride which is ordinarily added to food as a condiment is so large that the amounts of sodium and chlorine present in the various foods in their natural condition become usually of little practical consequence. But when there is profuse perspiration the water which leaves the body through the skin carries with it so much sodium chloride that it may become advan-

tageous to replace this loss either by taking a little salt with the drinking water or by adding a little extra salt to the food.

Among animals, the herbivora require salt while the carnivora do not, the latter obtaining sufficient salt for their needs from the flesh, and more especially from the blood, of their prey.

Sodium salts occur abundantly in the blood and other fluids of the animal body and in much lower concentration in the tissues. Potassium salts, on the other hand, occur to a greater extent in the soft solid tissues — in the corpuscles of the blood, the protoplasm of the muscles and other organs, and also in the highly specialized fluids which some of the glandular organs secrete, notably in milk. A conspicuous function of the salts in the tissues is the maintenance of the normal osmotic pressure, but solutions of different salts of equal osmotic pressure are for other reasons not interchangeable, and it is not possible to replace successfully the potassium in the cell by an equivalent amount of sodium.

Attention is frequently called to the fact that sodium chloride is the only salt which we seem to crave in greater quantities than occur naturally in our food, and that we share this appetite with the herbivorous animals. Bunge held that this is because a high intake of potassium (as furnished by most vegetable foods) tends to increase sodium elimination. Bunge tested this theory upon his own person by taking potassium phosphate and citrate, which was found to increase the elimination of sodium chloride; and concluded that while one might live without the addition of salt to the food even on a diet largely vegetarian, yet without salt we should have a strong disinclination to eat much of the vegetables rich in potassium, such as potatoes. While Bunge's explanations may not be entirely adequate in detail, there seems to be little doubt as to the correctness of his main deductions. They are, in general, confirmed by the more recent work of Miller (1923, 1926).

When no chloride is taken, the rate of chloride excretion falls rapidly to a point where the daily loss is only a very small fraction of the amount ordinarily consumed and excreted. Thus in an experiment by Goodall and Joslin in which a healthy man was placed upon a diet adequate in protein and energy value but practically free from salt, the excretion of chlorine on each of 13 successive days was respectively: 4.60, 2.52, 1.88, 0.87, 0.69, 0.48, 0.46, 0.40, 0.26, 0.22, 0.22, 0.17, 0.17 grams.

Cetti in ten days of fasting excreted altogether 13.13 grams,

and Belli in ten days on a diet poor in salt lost 11.8 grams, of sodium chloride. In Benedict's study of prolonged fasting his subject lost 8.44 grams of chlorine (equivalent to 13.93 grams sodium chloride) during the first ten days, 2.13 grams chlorine during the second ten days, and 1.57 grams chlorine during the third ten days of the fast. (See Table 33 above.) Since the body is supposed to contain about 100 grams of sodium chloride, it will be seen that even when there was complete deprivation of salt for as long as thirty days, the total loss did not exceed one fifth of the amount estimated as usually present in the body.

### **Sodium, Potassium, Calcium, Magnesium**

The distribution of sodium and potassium in the body and some of their mutual relations in metabolism have been referred to in the section on the chlorides. The distribution and functions of calcium have been studied in greater detail than those of magnesium. It is estimated that over 99 per cent of the calcium in the body belongs to the bones, the remainder occurring as an essential constituent of the soft tissues and body fluids. Of the magnesium in the body about 71 per cent is contained in the bones (Lusk). The muscles contain considerably more magnesium than calcium; the blood contains more calcium than magnesium.

That the calcium ion is necessary to the coagulation of the blood has long been known and frequently cited as an example of the great importance of calcium salts to the animal economy. Equally striking is the function of these salts in regulating the action of heart muscle.

It is well known that heart muscle may be kept beating normally for hours after removal from the body when supplied, under proper conditions, with an artificial circulation of blood or lymph or a water solution of blood ash. Howell, Loeb, and others have studied the parts played by various salts. The sodium salts take the chief part in the maintenance of normal osmotic pressure and have also a specific influence. Contractility and irritability disappear if they are absent, but when present alone they produce relaxation of the muscle tissue. Calcium salts also, although occurring in blood in very much smaller quantity, are absolutely necessary to the normal action of the heart muscle; while if present in concentrations above normal, they cause a condition of tonic contraction ("calcium rigor"). There is there-

fore a balance which must be maintained between calcium on the one hand and sodium (and potassium) on the other. Thus it is found that the alternate contractions and relaxations which constitute the normal beating of the heart are dependent in part upon the presence of a sufficient but not excessive concentration of calcium salts, and in part upon the quantitative relationship of calcium to sodium and potassium, in the fluid which bathes the heart muscle. Other active tissues of the body doubtless have analogous requirements as to inorganic salts.

Osborne and Mendel (1918) found that healthy growing animals (rats) can adapt themselves to shortage of chlorine, sodium, potassium, or magnesium to a rather striking degree; but that calcium and phosphorus are always needed in relatively liberal amounts.

Regarding the adequacy of the ordinary intake to meet the specific requirements for sodium, potassium, calcium, and magnesium, it would seem that only in the case of calcium (among these) is it ordinarily necessary to take thought in the selection of food materials or in the planning of dietaries. The amount of sodium chloride usually added to food is more than sufficient to meet the sodium requirement of the body, even if the natural sodium content of the food be entirely disregarded. Potassium and magnesium are relatively abundant in a wide variety of plant and animal foods, so that an ordinary mixed diet, unless it consist too largely of highly refined food materials, will usually furnish a safe surplus of these elements. Dietaries entirely adequate in energy value and protein content may, however, contain too little calcium. Calcium requirement is therefore a question of much practical importance in human nutrition, and requires quantitative study, which will be taken up in Chapter XIV.

### Phosphorus, Silicon, Fluorine, Iodine

Mellor quotes the saying that "if the biographies of the elements could be written, that of *phosphorus* would be the most interesting of all," and Peters and Van Slyke cite fourteen ways in which compounds of phosphorus may function in the body. Without attempting an enumeration of the nutritional functions of phosphorus, attention may be called to the fact that the phosphorus compounds concerned in the processes of nutrition include derivatives of all the main groups of organic nutrients, as well as mineral phosphates both readily soluble and relatively insoluble.

Phosphorus may be a limiting or determining factor in human nutrition, in that of farm animals, or in the nutritional and evolutionary processes of nature. Both in the sea and on the land, the available supply of phosphorus may determine the growth, composition, and multiplication of plants and through them of animals also. The quantitative aspects as affecting human nutrition are discussed in Chapter XIV.

Bunge and Abderhalden have emphasized the parallelism between the phosphorus and calcium content of milk and the rate of growth of the young, in different species (Table 35).

TABLE 35. — RELATION OF MINERAL ELEMENTS OF MILK TO RATE OF GROWTH (Bunge and Abderhalden)

| SPECIES<br>•  | NO. OF DAYS<br>REQUIRED TO<br>DOUBLE THE<br>BIRTH WEIGHT | PERCENTAGE COMPOSITION OF MILK (Partial) |       |         |            |
|---------------|--|--|-------|---------|------------|
|               |  | Protein                                  | Ash   | Calcium | Phosphorus |
| Human . . .   | 180  | 1.6                                      | 0.2   | 0.02    | 0.02       |
| Horse . . . . | 60   | 2.0                                      | 0.4   | 0.09    | 0.06       |
| Cow . . . .   | 47   | 3.5                                      | 0.7   | 0.12    | 0.09       |
| Goat . . . .  | 22   | 3.7 •                                    | 0.78  | 0.14    | 0.18       |
| Sheep . . . . | 15   | 4.9                                      | 0.84  | 0.18    | 0.11       |
| Swine . . . . | 14   | 5.2                                      | 0.80  | 0.18    | 0.14       |
| Dog . . . .   | 9  | 7.4                                      | •1.33 | 0.32    | 0.22       |
| Rabbit . . .  | 6  | 14.4                                     | 2.50  | 0.65    | 0.43       |

The bones and teeth, which contain over 99 per cent of the calcium of the body, are estimated to contain about 90 per cent of its phosphorus. The calcium and phosphorus of the bones and teeth are now said to be present essentially as crystal structures corresponding to those in phosphate rocks, and to be capable of representation by a mineral formula such as: (1)  $\text{CaCO}_3 \cdot n\text{Ca}_3(\text{PO}_4)_2$ , in which the numerical value of  $n$  is between 2 and 3; or (2)  $6\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$ . The chemistry of the bone salts is fully discussed by Logan (1940).

Our skeletal structures also resemble the phosphate rocks in containing small and variable proportions of *silicon* and of *fluorine*.

Silicon, while it has been regarded by some as a regular constituent of the enamel of the teeth, we can probably dismiss along with aluminum from any serious consideration *pro* or *con* in our present study. Our bodies have evolved in, and we live in, an



environment so rich in aluminum and silicon compounds that it seems improbable that we should be injured by the small amounts of our usual incidental intake; or that we should need more than we regularly get incidentally.

Fluorine presents a different sort of problem. It is very unevenly distributed in nature, and the natural waters of some localities contain enough to have deleterious effects.

Mottled enamel, a disease of human teeth, has been found by Smith, Lantz, and Smith (1931) to be due to fluoride naturally present in the drinking water of the afflicted communities.

In view of the chemical relationships of *iodine* and fluorine, the contrast in the nutritional significance of their occurrence in drinking water is striking; for the minute amount of iodide in drinking water is of great importance as a chief (often, if not usually, the chief) source of iodine for the manufacture in the thyroid gland of the nutritionally essential substance thyroxine. Because of its importance, a separate chapter will be given to the study of the nutritional rôle of iodine and its relation to the prevention of goiter.

### Iron, Copper, Manganese, Zinc

*Iron*, like phosphorus, is a constituent of the chromatin substances which are often referred to as concerned with the most vital activities of the cells, and recently it appears that a small amount of iron has an essential relation to the oxidation-reduction functioning of cytochrome; but much the largest part of the iron in the body exists as hemoglobin in the blood. In round numbers, the blood constitutes only about 7 per cent of the weight of the body, but contains at least 70 per cent of its total iron. The hemoglobins in different species of vertebrates differ but little: all are iron-containing proteins constituting a considerable part of the red blood corpuscles and functioning as oxygen carriers in respiration. In some of the lower animals (Crustacea, Mollusca) *copper*-protein compounds called hemocyanins are present and perform a similar function.

Copper has recently been found to function in minute amounts in the nutrition of the higher animals, as is explained in Chapter XV, in which the functions of iron in nutrition, and its occurrence in food, will be taken up more fully because of their great scientific and practical importance.

*Manganese.* Serious disorders can be induced experimentally by artificial deprivation of manganese. It is still an open question, however, whether one is likely ever to meet a case of manganese deficiency except when deliberately induced by artificial procedures.

The presence of manganese in plants was reported before 1850. Reiman and Minot (1920) found it widespread in human as well as other animal tissues. Investigations in McCollum's laboratory and at the University of Wisconsin, while differing somewhat in detail, together established the fact that manganese is to be included in the list of nutritionally essential elements.

Daniels and Everson (1935) found that young born to mothers which had been artificially deprived of manganese died either at birth or soon after. Young from these mothers, on being transferred to mothers from the stock diet, likewise died within a few days in almost all cases, although a few were raised until weaning time. However, when young from stock mothers were given at birth to mothers on manganese-deficient diet, almost all of them survived until weaning and attained a weight at 28 days close to that of the stock animals. The high percentage of deaths among the young from the manganese-deficient diet was therefore apparently due to inherent weakness on the part of the young at birth, rather than to failure of lactation in the mother.

In general, plant foods which are high in iron appear also to be good sources of manganese. Bertrand and Rosenblatt (1922) found manganese present in all the plants which they examined, particularly in the chemically active organs such as the leaves and seeds. McHargue (1922) reported that chlorophyll was not manufactured in the leaves in the absence of manganese from the soil, although only traces were needed to cause the plant to grow normally. In general, animal materials contain much less manganese than plants. Richards (1930) found among animal tissues the greatest amounts in the liver, pancreas, kidney, and duodenum, smaller amounts being always present in the reproductive organs, and extremely small amounts in muscle and blood. Milk contains a small amount, which appears to be maintained nearly constant regardless of the diet of the cow (Kemmerer and Todd, 1931). When Skinner and coworkers (1931) added a manganese salt to the stock diet of their rats, greatest storage occurred in the shafts and heads of the bones, in the hide, and in muscles and

liver. Everson and Daniels (1934) found the manganese retentions of children to be increased with increased intake, the amount of manganese being varied in the diets by choice of different natural food materials.

While manganese undoubtedly is important in human nutrition, the amounts required are so small that it is doubtful whether manganese is ever in practice the first limiting factor in the diet. A food supply of adequate vitamin value and phosphorus and iron content would almost certainly contain sufficient manganese. By markedly increasing the intake, the manganese content of the body can apparently be increased slightly; but whether or not this is advantageous remains to be determined.

If the estimates of manganese requirement be compared with the amounts of manganese in typical foods (Appendix C) it will be seen that only a small fraction of the food calories need be taken in such forms as fruits, vegetables, and whole-grain cereals and breadstuffs to provide liberally for the manganese needed.

*Zinc.* Hubbell and Mendel inclined to the view that zinc is not merely an accidental factor in nutrition, but refrained from further conclusion. The investigations both of Hart and coworkers and of Bertrand indicate that zinc is essential. The evidence is reviewed by Hart and Elvehjem (1936).

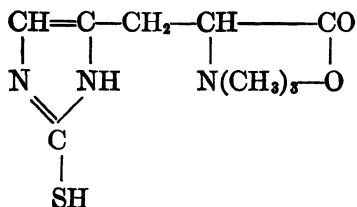
### Sulfur

While sulfur has long been known as a nutritionally essential element, modern developments have added greatly to our appreciation of the far-reaching significance of the various sulfur compounds in different phases of the chemistry of nutrition. The metabolism of sulfur is ably reviewed by Lewis.

Glutathione is a sulfur compound which undoubtedly plays a very important part in the oxidation processes of the body; and there is evidence that this may be true of other cysteine derivatives as well. Insulin also, as we have seen (Chapter V), is a sulfur compound. And still more recently vitamin B<sub>1</sub> has been found to contain sulfur; here in the form of the thiazole ring, not previously known to be concerned in nutrition. The structure of vitamin B<sub>1</sub> is discussed in Chapter XVIII.

Thioneine (previously called thiasine, ergothioneine, and sympectothion) has been isolated from blood and structurally

identified as the betaine of thiolhistidine (Newton, Benedict, and Dakin, 1927):



(Thioneine)

Its occurrence in the blood and its structural relationship to the nutritionally essential amino acid histidine add interest to the problem of its significance.

Thus sulfur plays a part as an essential element in several different substances which are active in the nutritional processes.

Plants absorb sulfates from the soil and use the sulfur in the synthesis of proteins. Minute quantities of inorganic sulfates may be taken by man in food and drink, but by far the greater part of the sulfur concerned in metabolism enters the body in organic combination and, so far as known, chiefly as protein. To a great extent the metabolism of sulfur may be regarded as a part of the protein metabolism, and in many respects the metabolism of sulfur tends under normal conditions to run parallel with that of nitrogen. In a series of ten experiments (each of 3 to 5 days' duration) upon man, in which the food consisted of bread and milk in varying amounts and proportions, the percentage absorption from the digestive tract was nearly the same for the sulfur as for the nitrogen of the food, and the excretion of the end products ran so closely parallel that in every case in which the body stored nitrogen it also stored sulfur, and vice versa (Sherman, 1902). Such parallelism, however, is not always found (Fay and Mendel, 1926; Lewis, 1924).

It is well known that individual proteins show relatively much greater differences in sulfur than in nitrogen content, so the ratio of nitrogen to sulfur varies widely, as is shown by the following examples selected from the data for pure proteins compiled by Osborne (Table 36).

Thus, while many proteins approximate the usually assumed average of 16 per cent nitrogen and 1 per cent sulfur, there are considerable deviations from this ratio in both directions.

TABLE 36. — NITROGEN AND SULFUR IN TYPICAL PROTEINS

| KIND OF PROTEIN          | NITROGEN        | SULFUR          | RATIO OF NITROGEN TO SULFUR |
|--------------------------|-----------------|-----------------|-----------------------------|
|                          | <i>per cent</i> | <i>per cent</i> |                             |
| Legumin . . . . .        | 18.04           | 0.385           | 46.9 : 1                    |
| Zein . . . . .           | 16.13           | 0.600           | 26.9 : 1                    |
| Edestin . . . . .        | 18.69           | 0.88            | 21.2 : 1                    |
| Gliadin . . . . .        | 17.66           | 1.027           | 17.2 : 1                    |
| Leucosin . . . . .       | 16.80           | 1.280           | 13.1 : 1                    |
| Casein . . . . .         | 15.78           | 0.80            | 19.7 : 1                    |
| Myosin . . . . .         | 16.67           | 1.27            | 13.1 : 1                    |
| Serum globulin . . . . . | 15.85           | 1.11            | 14.3 : 1                    |
| Egg albumin . . . . .    | 15.51           | 1.616           | 9.6 : 1                     |

Under ordinary conditions, however, it is the proportion of sulfur to the total protein of the food which determines the ratio of sulfur to nitrogen available for nutrition. Sulfur and protein have been determined in most staple foods, of which the data in Table 37 are representative.\*

Taking these figures as typical, it would appear that in those staple foods which contribute the greater part of the protein of the diet, the ratio of protein to sulfur does not differ greatly, and that in most cases of ordinary mixed diet there would be consumed not far from 1 gram of sulfur in each 100 grams of protein. We may therefore expect that in health and on an ordinary diet

TABLE 37. — SULFUR CONTENT OF FOODS IN PERCENTAGE OF THEIR PROTEIN CONTENT

| FOOD MATERIAL                   | SULFUR IN PERCENTAGE OF TOTAL PROTEIN |
|---------------------------------|---------------------------------------|
| Lean beef . . . . .             | 0.95-1.00                             |
| Eggs . . . . .                  | 1.4                                   |
| Milk . . . . .                  | 0.95-1.09                             |
| Wheat flour, crackers . . . . . | 1.15-1.29                             |
| Entire wheat . . . . .          | 1.30                                  |
| Oatmeal . . . . .               | 1.55                                  |
| Beans . . . . .                 | 0.69-1.00                             |
| Peas . . . . .                  | 0.80-0.94                             |
| Potatoes . . . . .              | 1.07                                  |

\* In the data here given, nitrogen and sulfur were determined in the same specimens. Average percentages of protein and sulfur in nearly all important food materials may be found in Appendix B and Appendix C, respectively.

the sulfur requirement will usually be covered when the protein supply is adequate.

It is chiefly the oxidation (to sulfate ion) of the sulfur taken into the body as protein which gives rise to the problem of fixed-acid elimination in acid-base balance.

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## CHAPTER XIII

### NUTRITIONAL ASPECTS OF ACID-BASE BALANCE

NOTWITHSTANDING the large amounts of acid produced in metabolism, the reaction of the blood plasma normally remains remarkably constant and slightly alkaline, between about pH 7.33 and pH 7.51 (Earle and Cullen, 1929); in healthy individuals, forced breathing or strenuous exercise may cause a departure from these limits for short periods of time; but about pH 7.0 to pH 7.8 is the extreme range reported compatible with life.\*

#### Buffers of the Blood

One reason that the blood can receive such large amounts of the acid products formed by the cells with so little change in its "reaction" (hydrogen ion activity, pH) is that it is highly buffered. Quantitatively the most important buffer systems of the blood are those of the proteins and their alkali salts. As has previously been pointed out, proteins are amphoteric substances capable of reacting either as acids or as bases. Since the chief proteins of the blood have isoelectric points more acid than the medium in which they occur, they are presumably present at this hydrogen ion activity as buffer systems made up of the protein in its acid form (HP), with an excess of its alkali salt ( $B^+P^-$ ). Van Slyke states that about one tenth of the buffer power of the blood is attributable to the plasma proteins, much the greater part of the rest being due to hemoglobin. This is partly because of the higher concentration of the latter (human blood contains in 100 cc. about 13 to 16 gm. hemoglobin and 3.5 to 4.0 gm. plasma protein), but also because at the hydrogen ion concentration of the blood, the *buffer value* of hemoglobin is more than twice as great as that of plasma proteins (Hastings *et al.*, 1924).

Hastings and Eisele (1940) found the acid-base balance of the blood of a normal adult at rest "to vary (in a regular, not random manner) during a 24-hour period due to . . . (a) a rise in the

plasma  $\text{HCO}_3$  associated with the ingestion of food; (b) an increase in the  $\text{CO}_2$  tension of the blood associated with sleep."

Phosphates in the blood form buffer mixtures made up of the acid ( $\text{H}^+\text{B}^+\text{HPO}_4^-$ ) and its salts  $\text{B}^+\text{B}^+\text{HPO}_4^-$ . A buffer system is most effective at a hydrogen ion activity approximating the dissociation constant of the buffer acid. Since the dissociation constant (pK) of monobasic phosphate at physiological salt concentrations is about 6.8, the reaction of the plasma is favorable for the buffer activity of the phosphates. However, their concentration in the blood is so low that their buffer effect here is overshadowed by that of the proteins.

### Elimination of Carbonic Acid

The bicarbonate-carbonic acid system has little buffer effect since, in solutions of the same ionic strength as blood plasma, the maximum buffer efficiency is observed at about pH 6.1, far from the pH of the plasma. Nevertheless, the blood bicarbonate has a special significance in the maintenance of a constant hydrogen ion activity within the body, since, because its acid is volatile, the large store of base combined in this way is available for the neutralization of acids other than carbonic. On the other hand, any base which is present in excess of acids other than carbonic takes the form of bicarbonate. Because of these properties the bicarbonate of the blood has been called the *alkaline reserve*. It is considered by Peters and Van Slyke (1931) as reflecting more or less closely the reserve of available alkali present in the body as a whole.

Of the acids which, in the normal process of metabolism, are continually formed in the cells, carbon dioxide is quantitatively by far the most prominent. The means by which it is transported from the cells to the lungs to be eliminated, without marked change in the hydrogen ion activity of the blood, are of special interest. To some extent, carbonic acid, like any other acid, is neutralized by the blood buffer systems which have been discussed. In addition, hemoglobin is rather uniquely adapted to function as a carrier of carbon dioxide as well as of oxygen. In the lungs, where the oxygen tension is high, hemoglobin combines readily with oxygen to form oxygenated hemoglobin; this in turn gives up its oxygen when it reaches the tissues, where the oxygen tension is low. Since the oxygenated form is a distinctly stronger acid than the reduced form, the change in the state of oxidation of

hemoglobin which occurs at the tissues is similar to the removal of acid. Less base being required to neutralize the weaker acid (reduced hemoglobin), more is made available to react with the increased amount of carbonic acid which is being received simultaneously by the blood. On the other hand, at the lungs, where hemoglobin is reoxygenated, the effect upon the bicarbonate of the blood is similar to the addition of acid, and results in an increased formation of carbon dioxide which is excreted.

Conversely, the tendency to increased acidity resulting from the higher tension of carbon dioxide in the tissues causes an increase in the dissociation of oxygenated hemoglobin into oxygen and hemoglobin, *i.e.*, tends to increase the liberation of oxygen in the tissues. Thus, the formation of oxygenated hemoglobin in the lungs directly increases the excretion of carbon dioxide while the increased tension of carbon dioxide in the tissues increases the liberation of oxygen from combination with hemoglobin.\*

Recently, Roughton (1935) and others have stressed the possibility that hemoglobin may combine chemically with carbon dioxide, the compound (or compounds) thus formed, *carbhemoglobin*, aiding significantly in the carrying of carbon dioxide in the blood.

Another constituent of the red blood cells, an enzyme (*carbonic anhydrase*) which accelerates the dehydration of carbonic acid to carbon dioxide and the reverse conversion of carbon dioxide to carbonic acid, assists in the interchange of gases both in the lungs and in the tissues.

From the discussion thus far, it appears that the cells of the blood are richly buffered; the plasma, on the other hand, only poorly buffered. Van Slyke and his coworkers have shown, however, that under physiological conditions, the buffering ability of the cells is shared with the plasma by an exchange of ions known as the *chloride shift*. There is also evidence that other anions to which the cell membranes are permeable, such as sulfate and phosphate, may undergo a similar redistribution with changing concentrations of carbon dioxide, but their influence is relatively slight because of their much lower concentration.

A very important factor in the elimination of carbon dioxide and in the regulation of the acid-base balance of the body is the fact that there is a tendency for the respiratory process to hold the tension of carbon dioxide in the blood nearly constant. Both

the depth and the frequency of breathing are supposed to be under the control of the so-called *respiratory centers* in the brain, the activity of which is most directly influenced by the pH of the cells in the center (Gesell, 1925). An increased production of carbon dioxide as the result of accelerated metabolism, during exercise, for example, tends to lower the pH of the blood, and consequently, of the cells in the respiratory center. This change stimulates breathing so that more carbon dioxide is eliminated by the lungs, and the hydrogen ion activity and the carbon dioxide tension of the blood are restored toward normal. According to Henderson (1928), "it is the changing ventilation of the lung that most directly controls the acid-base equilibrium of the blood."

### Elimination of Fixed Acids

In addition to carbonic acid, which is volatile and therefore readily eliminated from the lungs, non-volatile or fixed acids are also produced in metabolism and must be excreted. This involves two main steps: the immediate reaction of the acids with the bicarbonates and buffer salts of the blood or tissues; and the subsequent elimination of these acids as salts by the kidneys.

The action of the bicarbonates and buffers of the blood in neutralizing these acids immediately as they are formed is indeed a most important safeguard for the organism; but it is only a temporary measure, as the salts of the fixed acids formed in this way remain to be excreted and their removal from the blood involves the simultaneous removal of basic substances as well. In itself, this would tend to reduce the bicarbonate concentration or the alkali reserve of the blood and to result in the loss of fixed base from the body. It is here that ammonia and the buffer action of the salts of the urine play an important rôle.

The kidney has the ability to form (from nitrogenous "waste" products of metabolism) ammonia, which combines with the acid to be excreted, replacing in part the fixed bases, which latter are returned to the blood in the form of bicarbonates.

In contrast to that of the blood, the hydrogen ion activity of the urine varies rather widely, from about pH 4.8 to about pH 8.4, depending upon the proportions of acid and basic products formed in metabolism and upon the amounts and proportions of these products which are removed from the blood through other channels (particularly the lungs and the gastrointestinal tract). Strong

acids such as hydrochloric and sulfuric cannot exist as such, to any appreciable extent, in solutions of the hydrogen ion activity of the urine and must therefore be excreted with their chemical equivalent of base. On the other hand, weaker acids, such as monobasic phosphate and some of the organic acids of the urine, act as effective buffers in acid urine, an increased acidity of the urine resulting in the excretion of a larger fraction of the buffer in the acid form. For example, at pH 7.4 in the blood, about 80 per cent of the total inorganic phosphate is in the dibasic form, while at pH 4.8 in extremely acid urine, about 1 per cent is in the dibasic form, the remainder being in the monobasic form. In other words, by excreting a urine of pH 4.8, the body is able to conserve about two fifths of the base which had been combined in the blood with the excreted phosphate.

### Excretion of Organic Acids

It is comparatively rare under normal conditions that a freely chosen human diet gives rise to any considerable preponderance of basic products in metabolism. However, when this occurs, or when there is a temporary loss of acid as during active gastric secretion, the kidney assists in the maintenance of a constant hydrogen ion activity within the body. The urine becomes alkaline, with the result that the phosphate combines with relatively more fixed base. The excretion of ammonia practically ceases, fixed base being used instead for the neutralization of organic and inorganic acids. A large part of the excess base is eliminated as bicarbonate. The organic acids of the urine are of practically no significance as buffers under these conditions, since at the pH of the blood they exist almost wholly as neutral salts, and consequently can combine with little more base even at a more alkaline pH. In many cases of alkali excess, however, it has been noted that there is an increased formation and excretion of organic acids, these additional quantities of acid removing from the body their equivalent of fixed base. Citric acid has been studied particularly in this connection, but it is said that lactic, beta-hydroxybutyric, hippuric, and other organic acids may also occur in larger amounts when the urine is more alkaline. It has been found (Sherman, Mendel, Smith, and Toothill, 1936) that the urinary excretion of citrate for any given individual tends to parallel closely from day to day the pH of the urine, rising with an increase

in alkalinity, regardless of the nature of the dietary change which caused that increase. Under usual dietary conditions, the excretion of citric acid varies from about one quarter of a gram to one gram, but when sodium bicarbonate is taken by mouth, more than two grams of citrate may be present in the urine. It appears, therefore, that citric acid, which the body can synthesize, may have some importance in conserving the fixed acid of the body when there is an excess of basic elements to be eliminated. What other significance citric acid may have in the metabolic processes is still uncertain. Finally, it must be emphasized that the amount of base which is removed in combination with the organic acids when the urine is alkaline is only a small fraction of that which may be eliminated as bicarbonate.

Following is a list of some of the organic acids which have been found in normal human urine, with their ionization constants and approximate figures for the amounts excreted daily under usual conditions. The list is incomplete and the data in most cases only semi-quantitative because of the lack of satisfactory analytical procedures for identifying and determining specifically the individual acids.

| ACID                          | DISSOCIATION CONSTANT   | NORMAL EXCRETION |
|-------------------------------|---|------------------|
|                               | $K_a$   | Mg. per Day      |
| Citric . . . . .              | $\left\{ \begin{array}{ll} K_1 & 8.3 \times 10^{-4} \\ K_2 & 4.1 \times 10^{-5} \\ K_3 & 3.2 \times 10^{-6} \end{array} \right\}$ | 200-1200         |
| Uric . . . . .                | $1.5 \times 10^{-6}$  | 200-1000         |
| Hippuric . . . . .            | $2.2 \times 10^{-4}$  | 100-1500         |
| Lactic . . . . .              | $1.3 \times 10^{-4}$  | 70-100           |
| Formic . . . . .              | $2.1 \times 10^{-4}$  | 10-100           |
| Oxalic . . . . .              | $3.8 \times 10^{-2}$  | 10-50            |
| $\beta$ -hydroxybutyric . . . | $3.9 \times 10^{-5}$  | 0-75             |

### Influence of Diet

The normal fluctuations of fixed acid production in healthy man depend upon the diet. If the proportions of fat, carbohydrate, and protein are so unbalanced as to upset the normal ketogenic-antiketogenic relationship, the body may be flooded with organic acids arising from incomplete oxidation of fat. This is the same state that occurs in diabetic acidosis. Such a ketosis is also common in starvation and is artificially produced for therapeutic purposes by a ketogenic (high fat, low carbohydrate) diet.



It is, however, presumably rare in normal individuals on self-chosen diets and is not what we are concerned with in this discussion.

Since the non-volatile end products of normal metabolism are almost entirely inorganic in nature (with the exception of urea, which is neutral in reaction), an approximate estimate of the potential acid- or base-forming properties of a food may be made from a consideration of the amounts and proportions of the inorganic elements which it contains. A large number of foods were analyzed and their balance of acid-forming and base-forming elements computed by Sherman and Gettler (1912). For this calculation, the amounts of chlorine, phosphorus, and sulfur present were expressed as the equivalent number of cubic centimeters of normal acids; the amounts of calcium, magnesium, potassium, and sodium present were expressed as the equivalent number of cubic centimeters of normal alkali; and the excess of acid or base was obtained by difference. Typical results are shown in Tables 38 and 39.

TABLE 38. — FOODS IN WHICH ACID-FORMING ELEMENTS PREDOMINATE

| Food (Edible Portion)                  | APPROXIMATE POTENTIAL ACIDITY<br>(cc Normal Acid) |                  |
|--|---|------------------|
|  | Per 100 Grams                                     | Per 100 Calories |
| Beef, clear lean . . . . .             | 12  | 10               |
| round steak . . . . .                  | 11  | 7                |
| Eggs . . . . .                         | 11  | 7                |
| Oysters . . . . .                      | 15  | 30               |
| Oatmeal . . . . .                      | 12  | 3                |
| Rice . . . . .                         | 9   | 2                |
| Wheat, entire . . . . .                | 12  | 3                |
| Wheat flour . . . . .                  | 9   | 2                |
| White bread, made with water . . . . . | 6   | 2                |

It should be emphasized that this method of calculation was adopted for the sake of simplicity and that its simplifying assumptions are professedly only approximately correct. For instance, phosphorus is calculated as a dibasic acid, although we have pointed out above that at the pH of the blood it combines with about 1.8 equivalents of base. The assumption that the sulfur is completely oxidized and is neutralized entirely by inorganic base is also only an approximation; and the organic acids and carbon

dioxide are entirely omitted from these calculations of the balance of acid-forming and base-forming elements of the food.

Notwithstanding these limitations, the results calculated from the mineral analyses of foods have in practice shown a general relationship to the effects of the foods on the acid-base balance of the body, as indicated by the urinary acidity and ammonia, except in those cases in which the food contains a considerable amount of some organic acid which is not oxidized in the body. The foods of which this is known to be true are omitted from the accompanying tables. The mineral contents of commercial fats, sugars, and starches are too low for these to have any significant effect upon the acid-base balance, so they also are omitted from these tables.

TABLE 39. — FOODS IN WHICH BASE-FORMING ELEMENTS PREDOMINATE

| Food (Edible Portion)     | APPROXIMATE POTENTIAL RESERVE<br>ALKALINITY (cc. Normal Alkali) |                  |
|---------------------------|---|------------------|
|                           | Per 100 Grams   | Per 100 Calories |
| Apples . . . . .          | 3   | 6                |
| Bananas . . . . .         | 8   | 8                |
| Beans, dried . . . . .    | 10  | 3                |
| string, fresh . . . . .   | 5   | 13               |
| Beet, fresh . . . . .     | 10  | 25               |
| Cantaloupe . . . . .      | 7   | 18               |
| Carrots . . . . .         | 14  | 30               |
| Citron . . . . .          | 9   | 3                |
| Dates . . . . .           | 9   | 3                |
| Lemon or juice . . . . .  | 4   | 10               |
| Olives . . . . .          | 45  | 18               |
| Onions . . . . .          | 1   | 2                |
| Orange or juice . . . . . | 5   | 10               |
| Pears, fresh . . . . .    | 4   | 5                |
| Potatoes . . . . .        | 9   | 10               |
| Radishes . . . . .        | 5   | 23               |
| Rutabagas . . . . .       | 8   | 29               |
| Sweetpotatoes . . . . .   | 6   | 5                |
| Tomatoes . . . . .        | 5   | 24               |
| Turnips . . . . .         | 11  | 33               |
| Watermelon . . . . .      | 4   | 12               |

In experiments on man, Sherman and Gettler (1912) studied the effect of replacing the potato of a mixed diet by rice. It was found that 33 per cent of the calculated increase of fixed acid

from metabolism was accounted for by an increase in the urinary ammonia, and about 40 per cent by the increased titratable acidity of the urine. The remainder of the excess fixed acid may have been eliminated, in part at least, through the skin or in the feces, or may have been neutralized by the buffers of the blood with a corresponding increase in the carbon dioxide output and decrease in the reserve alkalinity of the body. In this experiment, the intake and output of phosphorus were approximately the same on the two diets. The increased acidity of the urine, therefore, implied an increased ratio of primary to secondary phosphate in the urine but not necessarily an increase in the amount of fixed base leaving the body. In the neutralization of sulfuric acid by means of phosphate, each molecule of the acid (representing one atom of sulfur oxidized to sulfate in protein metabolism) changes two molecules of secondary into primary phosphate. Whether or not this results in increased excretion of phosphate and therefore of fixed base (or only, as in the experiment just cited, in an altered ratio of primary and secondary phosphates in the urine) apparently depends upon a complex combination of conditions not yet fully understood.

Many fruits and fruit juices contain considerable amounts of organic acids, of which citric acid is perhaps the most familiar. Most of these are di- or tribasic acids and exist in the fruits partly as potassium acid salts and partly as free acids. As eaten, these fruits have an acid reaction; whether they are potentially acid or potentially basic in metabolism depends upon the extent to which the organic acid radicles are oxidized in the body, with the formation of potassium bicarbonate. Thus tomatoes, oranges, pears, peaches, apricots, and pineapples tend to diminish the acidity of the urine; while, on the other hand, cranberries, plums, and prunes usually increase urinary acidity. The explanation for the behavior of this latter group has been found in the presence in these fruits of relatively large quantities of quinic acid, which, instead of being oxidized completely in the body, is converted into hippuric acid which appears in the urine. It has been found that when human beings take by mouth as much as forty grams of citric acid (more than it seems likely that they would ingest per day in their food), less than 5 per cent of the acid appears in the urine (Sherman, Mendel, Smith, and Toothill, 1936). Less is known of the fate of malic acid in the human organism, although cats, rabbits, and dogs can destroy it to a large extent. Human tissues appar-

ently have almost no ability to oxidize tartaric acid. When tartrates or grape juice are taken by mouth, however, only a small fraction of the acid reappears in the excreta, the greater part being destroyed by the microorganisms in the gastrointestinal tract. The observation that the ingestion of grape products may, under favorable conditions, decrease the urinary acidity has been explained by the absorption of potassium bicarbonate which is a probable end product of bacterial action on the potassium acid tartrate of the grape.

Oxalates occur in small amounts in a great many fruits and vegetables, and in very considerable quantities in certain plants, including beet greens, spinach, and sorrel. Little is known concerning the ability of the body to metabolize oxalate. Moreover, the insolubility of calcium oxalate throughout the digestive tract makes it questionable how much of the oxalic acid of the food is actually absorbed; and, conversely, oxalate in the food may greatly reduce the absorption of the food calcium (Fincke and Sherman, 1935). Oxalic acid in small amounts is a normal constituent of the blood and urine, and probably, like citric acid, it is formed in metabolism. These considerations make it impractical to discuss oxalate-rich foods from the point of view of their effect on the acid-base economy of the body.

The question, whether or not there is merit under ordinary conditions in so choosing the food that the acid-forming elements thus introduced into the body shall be balanced by equivalent amounts of base-forming elements, must, in the opinion of the writer, be regarded as still open at the present time (1941). Much the greater part of the research which bears upon the question has been directed toward the elaboration of the theory of the maintenance of acid-base balance *in the blood*. So brilliant have been the results that there has been a tendency to overlook the limitations. Relatively little attention has yet been given to the important findings of Rous (1925) that all or most of the body tissues are somewhat less alkaline than the blood plasma, while the organs in which the nutritional processes are particularly active are, or may be, frankly acid (around pH 5.6). Thus some of the most important parts of the body get only partially the benefit of the buffering capacity of the blood; and so may be more influenced by an excess of acid-forming elements in the food than the studies of the blood would seem to suggest.

The working-out of the problems of the buffers and the carbonic acid carrying capacity of the blood is an outstandingly brilliant triumph of physico-chemical research. This knowledge of the blood, however, does not solve the problems of acid-base balance in all the separate organs of the body, nor permit one to say with confidence whether the balance of acid-forming and base-forming elements in food is or is not of practical significance in human nutrition.

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## CHAPTER XIV

### QUANTITATIVE ASPECTS OF CALCIUM AND PHOSPHORUS NEEDS AND VALUES

THE GENERAL positions of calcium and phosphorus in the nutritional process having been sketched in Chapter XII, the present chapter will deal with such quantitative questions as the amounts of each of these elements needed, and the amounts most desirable, in normal nutrition under different conditions, the amounts furnished in ordinary food supplies, and the relative values of individual articles and types of food as sources of calcium and of phosphorus.

Inasmuch as it has been found that simple salts of these elements can serve satisfactorily to supply them even to the rapidly growing body, and as it is believed that many of their complex organic compounds are largely changed to simple inorganic forms in the digestive tract before absorption, there is not now much disposition to discriminate between organic and inorganic forms in studying the quantitative need. In the main, the quantitative study discusses the amounts supplied by the food and taken in and given out by the body, without subdivision as to form of chemical combination; and such will be our plan throughout the greater part of this chapter. When, however, the general quantitative base-line and framework have been established in this way, it may be worth while to investigate articles and types of food more rigorously as to their individual values as sources of calcium and phosphorus, as illustrated briefly in the latter part of the chapter.

#### Method of Determining the Amounts Required

The method which permits of the most direct approach to the question of the amount of calcium or phosphorus needed in human nutrition is that of the *balance experiment* conducted upon the same general plan as in the case of the nitrogen balance experiments discussed in Chapter XI in connection with the study of the quantitative aspects of the protein metabolism and protein requirement.



Since both calcium and phosphorus after being metabolized in the body are excreted very largely by way of the intestine, and since the proportions of eliminated calcium or phosphorus leaving the body by way of the two chief paths of output (the kidneys and the intestine) may vary widely, no conclusions regarding bodily need of calcium or of phosphorus should ever be attempted except from experiments which include quantitative determinations in both urine and feces.

Hence, also, it would be seriously misleading to consider the amount of calcium or phosphorus found in the urine alone as a measure of the amount which the body had absorbed; or the amount in the feces as a measure of what had escaped utilization. It must be kept clearly in mind that *after utilization* a large part of the phosphorus and a still larger part of the calcium is likely to be eliminated through the intestine instead of through the kidneys.

Maintenance requirements of adults are studied by determining the balance of intake and output on diets of normal character but of low calcium or phosphorus content until one finds the minimum amount of calcium or phosphorus which will just permit of the maintenance of an equilibrium of intake and output of the element in question. If instead of exact equilibrium there is a small negative balance, the total output may be taken as indicating approximately the maintenance requirement. (The extremely small amount lost through the skin is not usually measured.)

In studying the calcium and phosphorus requirements of growth, balance experiments are also made, but here the plan which gives the most significant results is to find the intake which will support an optimal rate of storage of the element in question in the growing body. In the evolution of human anatomy and physiology there was doubtless "survival value" in the baby being born with flexible bones (an obvious advantage to both mother and child so far as the event of parturition is concerned, discussed further in the writer's *Food and Health*, 1934). But this means being born with calcium-poor bodies which must increase their *percentage*, not merely their amount, of calcium during their growth and development. Hence there is an accentuated need for calcium (and in lesser degree for phosphorus) over the need for body-building material in general. Thus the calcium and phosphorus requirements of growth are relatively higher than the protein requirement of growth. And unless the food furnishes the

relatively liberal calcium intake required for optimal calcium retention, the growing body will remain calcium-poor for an unduly long time.

In pregnancy also, optimal\* retention rather than merely equilibrium must be the guide to the estimate of optimal nutritional intake.

### The Calcium Requirement

Calcium constitutes a larger proportion of the body weight than does any other of the mineral elements. It is very unevenly distributed in the body, over 99 per cent of the total amount normally being in the skeletal system. It is also very irregularly distributed among the staple articles of food, many of which are extremely poor in calcium, while milk contains it in abundance, and green leaves in fairly liberal relation to their total solids or calories. A food supply may appear liberal and varied, and yet unless milk and green leaf vegetables are well represented it may be calcium-poor. Hence the "ordinary mixed diet" of Americans and Europeans, at least among dwellers in cities and towns, is probably more often deficient in calcium than in any other chemical element.

In adults there may be a long-continued loss of calcium without the appearance of symptoms because the losses from the blood and soft tissues may be replaced by calcium withdrawn from the bones. Bauer, Aub, and Albright (1929) and also Cannon (1932) have explained how in the organism which has been well supplied with food-calcium, the bones and especially their trabeculae serve

\* In this book, the adjective *optimal* or the noun *optimum* is intended to mean literally and strictly the *best*. In most cases we do not yet know just what level of nutritional intake is best or is needed by a person in order that he may do his best, or to what extent the latter may differ as a matter of individual variability among people apparently of the same category, or in what cases the amount of a given nutrient needed for best results is influenced by the level of intake of some other nutrient. Hence *optimal diet*, or even optimal intake of an individual factor, is in most cases an *ideal* or an *ultimate goal* which we cannot yet define in precise quantitative terms. The question has therefore been raised whether the use of the terms *optimal* and *optimum* should be suppressed. To the writer, discrimination seems better than suppression. Let these terms be used as frequently as seems desirable, but never loosely. It may frequently be desirable to second McCollum's teaching that in nutrition there is or may be an important difference between the merely adequate and the optimal; but it is never desirable to call a diet optimal merely because it is better than barely adequate. It shall also be remembered that optimal intakes may have upper as well as lower boundaries so that indiscriminating openhandedness in dietary allowances does not represent the best use of the science of nutrition.

as a calcium reserve, gradually drawn upon whenever there is a loss of calcium from the blood and soft tissues. It should also be noted that the form and arrangement of the trabeculae is at the same time very effective for the strengthening of the bones. They function *both* structurally and as stored material.

The book by Hoskins (1933) and other references at the end of this chapter will serve to put the reader who so desires in touch with the literature of the parathyroid glands and their effects upon calcium metabolism.<sup>4</sup> The present text assumes a normal endocrine constitution.

### Quantitative Study of the Maintenance Requirement

On account of the fluctuating distribution of calcium between urine and feces, conclusions regarding the calcium requirement can properly be drawn, as already explained, only from those experiments in which the amounts of this element in the food, in the feces, and in the urine have been directly determined. A compilation of such experiments was made in 1920, and the reported results calculated to a uniform basis of 70 kilograms of body weight. On this basis, 97 experiments (about equally divided between men and women subjects) show calcium outputs ranging from 0.27 to 0.82 gram and averaging 0.45 gram of calcium "per man per day." This estimate of average requirement was not changed by subsequent studies of the relation of vitamin D to calcium metabolism. It is also not significantly changed by the accumulation of further studies of the calcium balance as influenced by the level of intake.

*If all applicable data* published more recently than the above were now included, the average would apparently be raised somewhat. Leitch (1937, 1938) applying a different method of interpretation to the more recent data concludes that the average minimal adult requirement is 0.55 gram, and Owen (1939) in new experiments finds an average of 0.52 gram. Thus all available evidence consistently shows that the average minimal need of adults is about 0.5 gram of calcium per day.

Meanwhile, however, clinical studies of human experience and controlled experiments extended throughout the entire life-times of laboratory animals have brought abundant evidence that in the case of calcium the margin between the minimal requirement and the level of intake which induces the best permanent condition

of wellbeing is wider than we previously supposed. Hence dietary standards for calcium should now be set higher than has hitherto been customary. See also the sections on calcium in Chapters XXVIII and XXX beyond.

### **Requirements of Pregnancy and Lactation**

The calcium requirements of women are greatly increased by maternity. The need of an abundance of calcium for the rapidly growing skeleton of an infant is obvious. Before birth, and normally for several months after, this demand of the child is satisfied through the mother, whose need for calcium is thus greatly increased.

Lusk, citing the data of Hoffstrom, emphasized the importance of diet rich in calcium for pregnant women, especially during the last ten weeks of pregnancy when the fetus is storing calcium at a rapid rate. Blunt and Cowan summarize the results of several studies of calcium and phosphorus balances during pregnancy in their excellent book *Ultraviolet Light and Vitamin D in Nutrition*.

Some of the most carefully performed experiments have been planned in ways which render difficult or doubtful the exact interpretation of the observed balance of intake and output, especially when one or more factors of the intake have been allowed to vary from day to day; but it is safe to say that there is now much experimental evidence in support of emphasis upon the high calcium and phosphorus requirement of pregnancy. Usually from one third to one half more is allowed for pregnant women than for healthy adults generally.

### **Requirements of Normal Growth and Development**

In children, steady growth with a normally increasing percentage of body calcium requires a high calcium intake. Camerer, in summarizing a long series of investigations upon the food requirements of children at different ages, concluded that the amount of calcium received by the average nursing infant is just about sufficient to maintain a normal rate of growth, leaving little if any margin of safety; and Bunge, from a comparison of the calcium contents of different staple foods, pointed out that calcium more than any other inorganic element is likely to be deficient as the result of change of diet from milk to other forms of food. These fundamentally important facts should not be forgotten in the study of the

complexities added by later research, and recently assembled by Shohl (1939).

In the present book, space permits discussion of only so much of the evidence as seems to bear most directly upon the immediate problem. In 1922 an extended series of quantitative studies of the balance of intake and output of calcium in children of from 3 to 13 years of age was made by Sherman and Hawley. Although this antedated most of the work upon vitamin D, it is known that this vitamin was provided, because, both before and during the experiments, the children were kept much of the time in outdoor sunshine. Thus they received vitamin D through irradiation as well as through their food.

These experiments were carried out in four series, in the third and fourth of which a considerable part of the food calcium was supplied by a mixture of carrots and spinach. Inasmuch as spinach has been found (Fincke and Sherman, 1935) to be an unfortunate choice among green vegetables, at least as a source of calcium, the present paragraph cites only the data of the first two series of the Sherman and Hawley experiments, in both of which the calcium was supplied in food-forms of good nutritional availability. The purpose of the first series was to study the relation of calcium retention to age. Twelve children from 3 to 13 years old were studied as to balance of intake and output of calcium and phosphorus during a period of 9 days. All the children were normal and received a normal mixed diet including a fixed allowance of 750 grams of milk per child per day. This resulted in a nearly uniform calcium intake of about 1 gram per child per day. The calcium retention varied from 0.15 to 0.62 gram per day, increasing with the size of the child. Calculated to the basis of size the results show fair uniformity and indicate an average daily storage of 0.01 gram of calcium and 0.008 gram of phosphorus per kilo of body weight per day in these normally growing children. In the second series of experiments three of the children who had served as subjects during the first series were kept under continuous control and observation with quantitative determination of intake and output of calcium for much longer periods, the calcium intake being varied by systematic changes in the amount of milk in the diet. In part, the results are summarized in Fig. 24. At intakes of 0.4–0.45 gram per day, all showed positive calcium balances but low rates of retention. An increase of intake to

0.7–0.75 gram per day had a markedly favorable effect upon retention in all cases. A further increase of intake to about 1.0 gram per day apparently served chiefly as insurance in the cases of K. C. and M. O.; but was evidently very directly utilized by E. C. to enable her normal process of calcification (strengthening of bones and development of teeth) to catch up with her age and size.

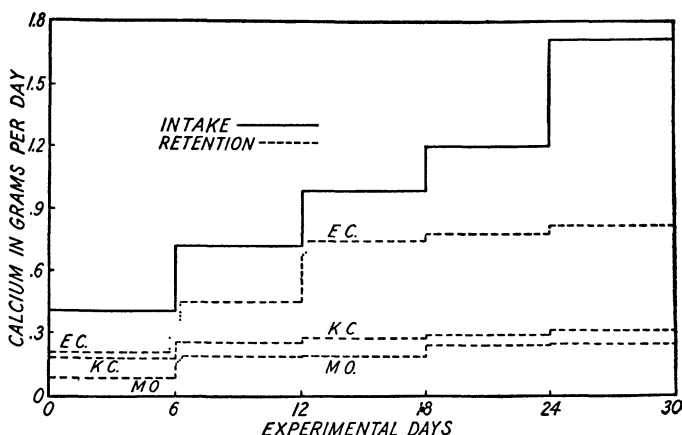


FIG. 24. — Relation of calcium retention to calcium intake in three girls: E. C., aged 12 years, weight 34 kilograms; K. C., aged 4 years, weight 18 kilograms; M. O., aged 5 years, weight 21 kilograms. In each experimental period of six days, the intake was essentially the same for each child. The graphs show (a) the level of calcium intake of all, and (b) the respective calcium retentions of each.

Increases of intake above 1.0 gram of calcium per day caused only a small further increase of retention in any of these cases.

In the light of the suggestion of Daniels and coworkers that the previous nutritional history of the child may influence its capacity for retention of calcium and thus the level of intake required for optimal retention in the individual case, the results with these three girls might perhaps be interpreted as meaning that the large capacity for retention shown by E. C. was due to a calcium-poor condition of the body (as well as to her greater size); and that M. O., who retained lower amounts and proportions at all levels of intake, may have been so nourished as already to have a body well stocked with calcium for her age and size; while K. C. may have been in an intermediate condition with regard to her previous dietary history. Probably E. C. represents the condition

of a large proportion of the children with whom one has to deal; so that the intake of about one gram of calcium per day which was found necessary to insure optimal retention in this case represents a desirable allowance to provide for individual differences and to ensure against ordinary fluctuations of efficiency in assimilation. Doubtless K. C. also represents a common type of case, able to gain even on an intake of 0.45 gram, but gaining much better on 0.75 gram and still better on an intake of 1.0 gram per day.

Outhouse, Mitchell, and coworkers at the University of Illinois have emphasized their difficulty in securing the retention by children of more than about one-fifth of the calcium fed, while Jeans and Stearns of the University of Iowa find higher percentages retained by a large proportion of children, as was also the case among the New York City children studied by Sherman and Hawley.

Whichever of these viewpoints we emphasize, the conclusion is the same: liberal calcium intake is needed by the body of the infant or child, which in order to develop to best advantage must retain a relatively larger amount of calcium than of other building materials, because normal development involves increase, not only of the amount, but also of the percentage of body calcium during growth.

At the time of the present writing the full accounts of the extended investigations of Jeans and Stearns and of Macy and coworkers still await publication, but the general trend of their findings, as briefly sketched by Shohl (1939) and kindly supplemented by personal communications from the investigators to the present writer, unquestionably support high standards of calcium intake. Noteworthy also is Jeans and Stearns' repeated emphasis upon the fact that liberal allowances of vitamin D are desirable but do not justify any slackening of emphasis upon the importance of a liberal supply of food calcium.

If the objective were merely to provide for positive calcium balances in children, this could be accomplished, as shown by Sherman and Hawley in 1922 and by Wang and coworkers in 1930, with intakes of only 0.4 to 0.46 gram of calcium per day; but to provide for such rates of retention as the newer knowledge of nutrition deems desirable calls for an average daily intake of about 1.0 gram. The fact that a special preperiod of extra high-calcium feeding may so stock the body with calcium that for a time there-

after optimal storage will not require so high an intake has a certain theoretical interest but little practical bearing upon our actual problem of the feeding of children as we find them. Of greater practical import is the evidence assembled by Leitch (1938) which seems to call for a decided advance in our standards of calcium content for the human body, and of the rates of retention needed to realize them.

From personal communications with Dr. Jeans, and independently with the late Dr. Todd, the writer has learned that without necessarily following all of the assumptions involved in Leitch's reasoning they did concur in its main conclusions: that present knowledge calls for considerably higher estimates of the normal calcium content of the properly developed human body than have been current hitherto; and that the attainment of these higher percentages requires (along with plenty of vitamin D) high standards of calcium intake for infants and for older children up to such time as they attain full "chemical maturity" and optimal adult skeletal development.

A digest and discussion of the different numerical estimates of supposedly normal calcium retentions of children at each stage of growth and development has recently been published (Sherman and Lanford, 1940, pages 131-136) and need not be repeated here.

Until such time as the precise numerical estimates of leading investigators can be brought into closer quantitative agreement, it seems best to use the round figure of 1.0 gram of calcium per day as a guide to ("standard" of) adequacy of intake for children of all ages. In household terms this is best accomplished by providing an average of about a quart of milk per child per day (not too dogmatically for "every" child, for an occasional child may have an idiosyncrasy which interferes; but, for general teaching and practice, an average of about a quart is better than any lower average).

We may be asked why we assume that something approaching maximal calcium retention is optimal, *i.e.*, does result in greatest benefit in the long run. The answer is that we do not "assume" it, it has been found by scientific research. Obviously, an experimenter who wishes to study the life-time effects of different nutritional regimes must use a subject whose natural life cycle is sufficiently shorter than his own to permit him to see the experiment through. Also, the species chosen to furnish the subjects which



are to serve as our "deputies" in experiments designed to throw light upon the problems of our human nutrition must be sufficiently similar to us in their nutritional chemistry. The laboratory rat qualifies excellently in both these respects for the study of the effects of different levels of calcium intake upon permanent nutritional wellbeing.

And it may now be stated in the light of findings of hundreds of full-life, successive-generation experiments with laboratory rats, that essentially the same liberal levels of calcium intake, from two to four times that of minimal adequacy, which best expedite the normal calcium retention of growth and development, also yield the best results in adult vitality, length of life, and vigor of offspring (Sherman and Campbell, 1935; Lanford and Sherman, 1938; Sherman, Campbell, and Lanford, 1939; Van Duyne, Lanford, Toepfer, and Sherman, 1941).

The question, What happens when the growing body gets only a lower level of calcium intake?, is best answered by experiments with rats whose bodies can be actually analyzed when desired, as described in several of the papers listed at the end of the chapter (*e.g.*, Sherman and MacLéod, 1925; Sherman and Booher, 1931; Campbell, Bessey, and Sherman, 1935).

For simplicity of illustration and convenience of discussion, either typical individual cases or the average data of series may here be cited; but it will be understood that in all cases conclusions are based upon consistent experience with large numbers of animals under carefully controlled conditions.

In one case, twin brother rats, alike at the beginning of the experiment, were placed: one on a diet of meat, wheat, and milk; the other upon a diet of the same meat and wheat but without the milk. The former grew and developed normally; the latter showed some stunting of growth and markedly poor skeletal development because of his less adequate diet (Fig. 25). In this case, the relative calcium contents of the two diets were approximately as 100 : 14.2. This difference in calcium contents of the diets was doubtless the chiefly significant factor in this experiment.

In another case twin sisters were mated with twin brothers, making two pairs in all respects alike at the beginning of the experiment and having exactly the same hereditary background. Both families were fed wheat-and-milk mixtures; but in one case in the proportion by weight of 2 parts of ground whole wheat

to 1 part of dried whole milk, in the other case in the proportion of 9 parts of ground whole wheat to 1 of dried whole milk. The difference in calcium content was again the most significant

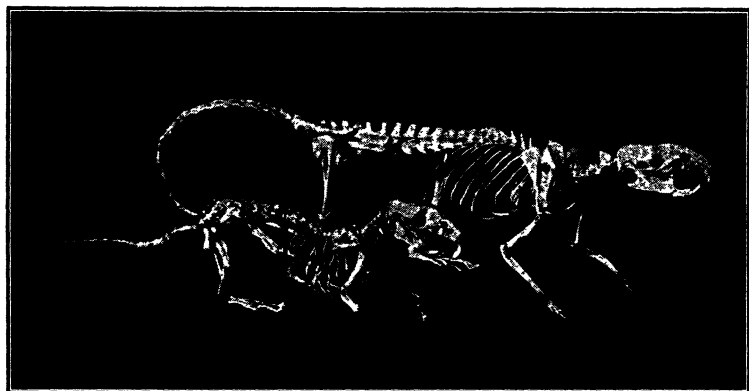


FIG. 25. — Twin brothers showing effects of different diets. See text. (Courtesy of the *Journal of Biological Chemistry*.)

difference between the two diets, but was not so pronounced in this case and correspondingly the effect was slower to appear. The original experimental animals showed only inconspicuous differences, and both families reared offspring; but in the second genera-

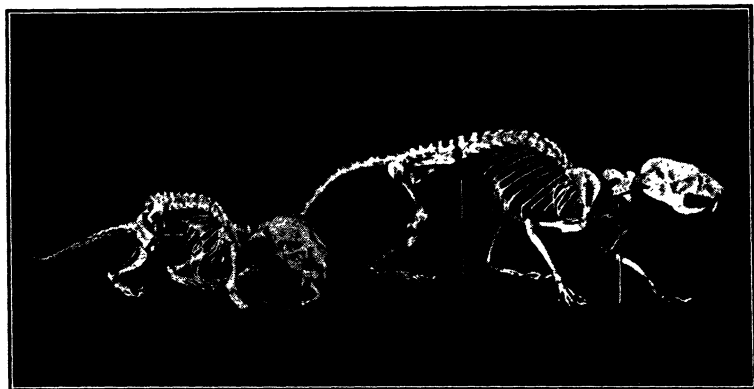


FIG. 26. — Cousins from families on different diets. See text.

tion (continued on the same diets as their respective parents) the difference in size and skeletal development was very similar to that which had appeared in the first generation of the experiment noted in the preceding paragraph. (See Fig. 26.) This is

but one of many cases in which it has been found that observations extended through more than one generation may be required in order to ascertain fully the influence of a difference in the food supply. In this case the calcium contents of the two diets were approximately as 100 : 36.8. Here also, calcium was doubtless the chiefly significant, though not the sole variable, factor.

On diets differing only moderately in composition (namely: Diet A, five sixths ground whole wheat to one sixth dried whole milk; Diet B, two thirds ground whole wheat to one third dried whole milk),\* rat families have thrived generation after generation, both diets being adequate and supporting normal nutrition (Sherman and Campbell, 1924), yet Diet B with its higher proportion of milk and consequent higher calcium content resulted in a more favorable rate of calcification of the growing bones so that chemical analyses of the bodies of representative animals at a given age showed, for example, 0.7 per cent calcium in those on Diet A, and 0.9 per cent calcium in those on Diet B. Inasmuch as Diet B was richer than Diet A in fat-soluble vitamins as well as in calcium, numbers of parallel experiments were made in which Diet A was separately enriched, on the one hand with fat-soluble vitamins by addition of cod liver oil; on the other hand, with calcium by addition of calcium lactate. The results (Table 40 based on experiments of F. L. MacLeod) showed that Diet A had already contained enough fat-soluble vitamin to enable the body to make use of the available calcium, and that what was needed here was a more liberal intake of calcium itself. The difference in calcium content between Diet B and Diet A was as 100 : 56.2.

TABLE 40. — AVERAGE CALCIUM CONTENTS OF 90-DAY-OLD MALE RATS ON DIFFERENT DIETS

| FOOD OF EXPERIMENTAL ANIMAL           | PERCENTAGE OF CALCIUM IN BODY |
|---------------------------------------|-------------------------------|
| Diet A . . . . .                      | 0.70                          |
| Diet B . . . . .                      | 0.90                          |
| Diet A plus cod liver oil . . . . .   | 0.69                          |
| Diet A plus calcium lactate . . . . . | 0.92                          |

In this case it was known that Diet A was relatively richer in phosphorus (and other mineral elements) than in calcium, and

\* In both cases distilled water was furnished *ad libitum* and sodium chloride (purified table salt) in the proportion of 2 per cent of the weight of wheat consumed.

therefore that enrichment of the calcium intake could be effected, without danger of incurring an unfavorable ratio of calcium to phosphorus or to other mineral elements, by the feeding of just enough of a pure calcium salt to equalize the proportions of calcium in the two diets. In human nutrition, the enrichment of the diet in calcium should normally be accomplished not by the use of calcium salts as such but rather by increasing the consumption of calcium-rich food, especially milk, which contains, along with its abundant calcium content, such proportions also of phosphorus and other mineral elements as to ensure improvement of the dietary in its mineral content as a whole.

It seems important to emphasize strongly the fact that on diets of fairly good nutritive value but containing less than the liberal

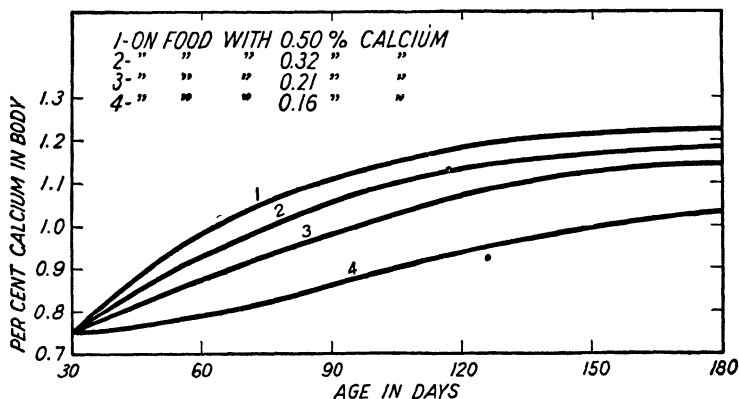


FIG. 27. — Percentages of calcium in the bodies of young rats in relation to age and to the calcium content of the food. In nutritive factors other than calcium the diets were of the same composition. (Courtesy of the *Journal of Biological Chemistry*.)

amount of calcium which is needed for the optimal retention of calcium during growth, there may be normal gains in height and weight and every appearance of good health as indicated by careful and thorough physical examination, yet the body (even though gaining calcium) may be calcium-poor because the *rate* of calcium retention is not as high as is needed to enable it to increase its percentage of calcium as rapidly as the *best* development of bones and teeth requires. And, as illustrated in Fig. 27 (based on experiments of L. E. Booher), the body may be delayed throughout the whole period of growth in bringing its calcium

content up to what would be fully normal for the age. In other words, growing children whose height, weight, and appearance are normal may have a calcium-poor condition of body which even the best physical examination cannot reveal, but which is revealed by the chemical evidence of the calcium balance experiments determined directly upon children of different ages and fully confirmed by analyses of the bodies of experimental animals. And the dietaries, resulting in this retardation of calcium retention and consequent calcium-poor condition of apparently well nourished individuals, were no poorer in calcium than many that are actually encountered in everyday human experience.

### The Phosphorus Requirement

#### Quantitative Study of the Maintenance Requirement

Since the excretion of metabolized phosphorus through the intestine is, in man, too large to be neglected and too variable to be allowed for by calculation, we can expect reliable data on phosphorus requirements from those experiments only in which the amounts of phosphorus are actually determined in food, in feces, and in urine. In such experiments it is found (as in the case of nitrogen) that the output obtained upon the experimental days is influenced not only by the food taken at the time, but also by the rate of metabolism to which the body had been accustomed on the preceding days. This is illustrated by the results obtained in a 12-day series of experiments upon a healthy man (Table 41).

TABLE 41. — PHOSPHORUS METABOLISM WITH DIFFERENT AMOUNTS OF PHOSPHORUS IN THE FOOD

| EXPERIMENTAL PERIOD |          | PHOSPHORUS PER DAY |              |              |              |              |
|---------------------|----------|--------------------|--------------|--------------|--------------|--------------|
| No.                 | Duration | In Food            | In Feces     | In Urine     | Output       | Balance      |
|                     |          | <i>grams</i>       | <i>grams</i> | <i>grams</i> | <i>grams</i> | <i>grams</i> |
| I                   | 3 days   | 0.40               | 0.45         | 0.70         | 1.15         | — 0.75       |
| II                  | 6 days   | 0.77               | 0.19         | 0.72         | 0.91         | — 0.14       |
| III                 | 3 days   | 1.51               | 0.50         | 0.99         | 1.49         | + 0.02       |

Here the output of phosphorus was greater in the first period with 0.40 gram in the food than in the second when the food furnished 0.77 gram, probably because the first period followed and was influenced by a preceding diet fairly rich in phosphorus,

whereas the output in Period II was influenced by the low-phosphorus diet of Period I. For the same reason, Period II offered favorable conditions for the establishment of equilibrium on a minimum diet, but the results show that in this case the subject was unable to reach equilibrium on 0.77 gram per day, the output averaging 0.91 gram. When the intake was increased to 1.51 grams, the output rose rapidly and averaged 1.49 grams. In this case the amount which would have been just sufficient for equilibrium evidently lay between 0.91 and 1.49 grams per day. By means of well-planned experiments or series of experiments it is possible to fix for a given individual much narrower limits within which the exact amount required for equilibrium must lie, and when it is known that the intake approximates this required amount, it is justifiable to regard the output as an indication of the normal maintenance requirement.

Study of the data of 95 such phosphorus balance experiments upon 21 subjects, 14 men and 7 women, has shown a range of 0.52 to 1.20 grams with an average of 0.88 gram phosphorus (2.02 grams  $P_2O_5$ ) per 70 kilograms of body weight per day. This is an average of findings as to actual requirement and should not be confused with dietary allowance or so-called dietary standards. The latter will be discussed in Chapter XXVII.

### Requirements of Normal Growth

In the experiments of Sherman and Hawley described above in connection with the discussion of calcium requirements for growth, it was found that optimal retention of phosphorus in the growing children of 3 to 13 years of age was not obtained until the intake reached from 1.16 to 1.46 grams of phosphorus per child per day, thus indicating that the child needs for optimal growth and development about one and one-half times as much phosphorus as is needed by a full-grown man for maintenance.

Additional studies have been conveniently summarized by Blunt and Cowan (1930).

### Ca/P Ratios

Especially since the common use of diets of distorted Ca/P ratios to produce experimental rickets, there has been something of a tendency to state Ca/P ratios in connection with normal nutrition also. Observations upon several species have indicated that

during rapid growth and calcification it is well that the food as a whole should have a Ca/P ratio somewhere between 1 and 2. When adult maintenance only is involved, the calcium requirement is lower, both absolutely and relatively to the phosphorus requirement. Thus the Ca/P ratio of the nutritional need is highest (widest) at the earliest ages; decreases with the attainment of adult status; then, in the case of the female, increases again in the latter part of pregnancy and in lactation.

The ratio of calcium to phosphorus in the body as a whole is normally increasing throughout the period of growth and calcification. In the normal adult mammal the Ca/P ratio of the whole body is a little under 2; and that of the bones is a little over 2.

Meigs and coworkers (1935), as the result of their very thorough experimental investigation of milch cows and study of previous work, have concluded: that the percentages of calcium and phosphorus contained in the fat-free bodies of mature normal cattle are relatively constant, and the Ca/P ratio, both in the whole body and bone, still more so; that keeping cows on low-calcium rations for long periods alters these relationships less than has often been thought (their long periods on calcium-poor foods changed the Ca/P ratio of the whole body only from 1.9 to about 1.8, for when calcium was lost phosphorus was lost also); and that previous reports of positive balances on low-calcium intakes and of retention of calcium and phosphorus in widely variable ratios are probably due to experimental errors.

Obviously when intakes of both elements are right, the ratio cannot be wrong.

### **Further Interpretation of the Calcium and Phosphorus Requirements**

**The requirements of adult maintenance.** Having at hand the data of about 100 cases each of reasonably comparable experiments designed to measure the maintenance requirements for protein, phosphorus, and calcium, respectively, in normal human nutrition, it was of interest to compare the variability of the individual observations in the three series of experiments (each representing the work of several different investigators) and the probable errors of the three mean results as computed by accepted statistical methods.\*

\* A summary of such methods, in so far as required for the simple statistical interpretation of experimental data such as here given, may be found in the Appendix.

The 109 experiments upon protein requirement show a mean of 44.4 grams, a standard deviation of 9.07 grams, a coefficient of variation of 21, and a probable error of the mean of  $\pm 0.58$  gram.

In 95 experiments upon phosphorus requirement the mean with its probable error is  $0.88 \pm 0.01$  gram, the standard deviation is 0.15 gram, and the coefficient of variation is 17.

The 97 experiments upon calcium requirement mentioned above give a mean result of 0.45 gram with a probable error of  $\pm 0.008$  gram and show a standard deviation of 0.12 gram and a coefficient of variation of 27.

In each of the three cases the data are sufficiently numerous and consistent so that the probable error of the mean is less than 2 per cent of its value, making it unlikely that any of these averages will be much changed by the accumulation of more experimental evidence of the same kind. That a more critical selection of the data would result in a somewhat lower estimate of the protein requirement has been pointed out in Chapter XI. That knowledge of vitamin D will materially lower the general average calcium or phosphorus requirement is not probable. As explained in discussion of calcium above, subsequent experiments confirm the 1920 averages here given.

The averages just cited indicate that the calcium requirement is a little over one hundredth of the protein requirement, and the need for a margin over actual requirement as a factor of safety would seem to be at least as great in the case of calcium as of protein. Hence, whatever the factor of safety adopted or margin allowed for individual variations, it would follow that a food supply, in order to furnish these essential nutrients in relative proportions corresponding to the needs of the body, should contain at least one hundredth as much calcium as protein. This is not usually the case in American dietaries, from the study of which it appears that most Americans carry a much larger nutritional insurance as regards protein than as regards calcium.

Protein requirement of 44.4 grams per 70 kgm. per day for normal maintenance means a daily exchange of one third of one per cent of the protein which the body contains.

Of phosphorus the corresponding exchange is one eighth of one per cent. That the rate of exchange of phosphorus is relatively lower than that of nitrogen (protein) is probably because the greater part of the phosphorus in the body (perhaps about



90 per cent) is in the form of relatively stable and insoluble bone material.

Of calcium the corresponding exchange is only one twentieth of one per cent of what the body contains — a still smaller proportion than of phosphorus because a much larger proportion of the total body calcium than of the total body phosphorus is contained in the skeletal system.

The calcium phosphate of the bones, while undergoing a less rapid exchange of material than the proteins and soluble phosphates of the soft tissues, is yet demonstrably undergoing metabolism as shown by the experiments of Chiewitz and Von Hevesy (1935) and others in which it has been found that radioactive phosphorus, when fed to full-grown normally nourished animals, enters largely into the composition of bone in exchange with the phosphorus previously there. Evidently there is a continuous, though relatively slow, metabolism in even the hardest mineral structures of the body.

Taking the requirements of normal maintenance as the basis of comparison, it appears that, among presumably typical dietaries actually observed, about 1 per cent were deficient in amount of protein, about 4 per cent in amount of phosphorus, and about 16 per cent were deficient in the amount of calcium which they contained. Some of these deficiencies are plainly due, at least in part, to the consumption of an insufficient amount of food. If in all cases of lower food intake the latter were increased to a total food value equal to 3000 Calories per man per day, none of the dietaries would then be deficient in amount of protein, only about 1 per cent would be deficient in phosphorus, but about 9 per cent would still be deficient in their calcium content. And if deficient from the point of view of the calcium requirement of the adult, they would be still more so when considered from the standpoint of the needs of growing children, as already explained.

Do other peoples thrive on lower calcium intakes than are indicated by observations and experiments in America and Europe? Probably not. It is true that the milk supply which is our main source of dietary calcium is very scanty in some other parts of the world, notably in the Orient, the Arctic, and among some (by no means all) of the native peoples of Africa. But in every case which has been carefully studied it appears, not that such peoples thrive

on less calcium but that they utilize sources of calcium which we neglect.

Adolph finds the calcium needs of Chinese about the same as we have here found, and makes clear that calcium is recognized as an important nutritional problem in China; while Hoh, Williams, and Pease (1934) have shown how a typical Chinese method of cookery brings into human nutrition a large amount of the calcium of bone, practically none of which is utilized in most American households. Kelly and Henderson (1930) found the calcium requirement of African natives essentially the same as that of Europeans and Americans. In the Arctic and in parts of Asia and Africa much bone-calcium is utilized even without the intervention of cookery — by simple mastication of the smaller bones and the soft ends and interiors of the larger ones. Also, in the Orient and in parts of Africa much larger use is made of green leaf foods than in America, and in certain regions not only do the people eat many coarser kinds of leaves than we do; but of the leaves which are too coarse even for them to eat, they eat the ashes.

### **Calcium and Phosphorus Contents of Typical Foods**

Since it is now apparent that a freely chosen food supply may not always furnish the needed amounts of calcium and phosphorus, it becomes important to know the calcium and phosphorus contents of foods. Simple averages for nearly all familiar foods will be found in the Appendix. For actual study in connection with the present text, there are given in Tables 42 and 43 the data for a limited number of typical foods to illustrate differences in quantitative distribution, and also to indicate what measures of precision attach to present averages for different foods and what coefficient of variation is inherent in each.

**Relative accuracy of our knowledge of calcium and phosphorus contents of foods.** The following statements, here based primarily upon the data summarized in Tables 42 and 43, are also significant of these foods as typical. In milk both the calcium and the phosphorus content is now well established and shown also to be relatively constant. For eggs, the analyses are less numerous and the averages have considerably higher probable errors, yet may be regarded as fairly well established. The higher coefficients of variation shown by the data for eggs (as compared with milk) may be largely due to differing proportions of white and yolk, especially

as the removal of edible portion for analyses has sometimes been by merely pouring it from the shell and sometimes by meticulous removal of every bit of the white. Lean beef (typical mammalian muscle) shows, as might be expected on biological grounds, a relatively constant phosphorus content. That the data for its calcium content are so much more variable may be due to errors of sampling and analysis. The calcium content of muscle is so low that the *relative* error in its analytical determination is apt to be high; and also the accidental inclusion of traces of bone or even of gristle

TABLE 42. — CALCIUM CONTENTS OF A FEW TYPICAL FOODS

| ARTICLE OF FOOD | NUMBER OF CASES | MEAN % Ca IN EDIBLE PORTION | PROBABLE ERROR (P.E.) OF MEAN | P.E. IN PERCENTAGE OF MEAN | COEFFICIENT OF VARIATION |
|-----------------|-----------------|-----------------------------|-------------------------------|----------------------------|--------------------------|
| Milk . .        | 274             | 0.118                       | $\pm 0.00037$                 | 0.3                        | 7                        |
| Eggs . .        | 36              | .058                        | $\pm 0.0010$                  | 1.7                        | 16                       |
| Lean beef .     | 45              | .013                        | $\pm 0.0007$                  | 5.4                        | 56                       |
| Almonds .       | 31              | .254                        | $\pm 0.0078$                  | 3.1                        | 26                       |
| Dry beans .     | 29              | .148                        | $\pm 0.0049$                  | 3.3                        | 26                       |
| Wheat . .       | 94              | .057                        | $\pm 0.0013$                  | 2.3                        | 32                       |
| Sweet corn .    | 17              | .009                        | $\pm 0.0004$                  | 4.4                        | 29                       |
| String beans    | 53              | .065                        | $\pm 0.0018$                  | 2.8                        | 31                       |
| Apples . .      | 95              | .007                        | $\pm 0.00027$                 | 3.9                        | 56                       |
| Dates . .       | 15              | .072                        | $\pm 0.0027$                  | 3.8                        | 22                       |
| Cabbage. .      | 72              | .045                        | $\pm 0.0013$                  | 2.9                        | 36                       |
| Kale . .        | 14              | .181                        | $\pm 0.0046$                  | 2.5                        | 14                       |
| Asparagus .     | 23              | .021                        | $\pm 0.00107$                 | 5.1                        | 36                       |
| Potatoes .      | 78              | .013                        | $\pm 0.0008$                  | 6.1                        | 82                       |
| Beets . .       | 31              | .026                        | $\pm 0.0011$                  | 4.2                        | 35                       |
| Carrots . .     | 50              | .042                        | $\pm 0.0010$                  | 2.4                        | 26                       |
| Parsnips .      | 13              | .057                        | $\pm 0.0013$                  | 2.3                        | 13                       |
| Turnips         | 29              | .051                        | $\pm 0.0019$                  | 3.7                        | 31                       |

will very unduly influence the calcium content of muscle. In Tables 42 and 43 there follow, as typical food seeds from diverse botanical families, almonds, dry beans, and wheat; then sweet corn as an immature seed, followed by the combined immature seed and succulent green pod of the snap or string bean; then in order, typical fruits, leaves, shoots, the potato (an enlarged underground stem), and roots.

As indication of the validity of the averages (means) here shown, note that for only a few of these foods is the probable error of the

mean as much as five per cent of its value; also that the coefficients of variation are generally low enough to give added confidence.

Average calcium and phosphorus contents of many other foods are tabulated in Appendix C.

In using all such averages it is to be remembered that because of natural variations any new sample may differ from the previously determined average, but this does *not* mean that the average is "meaningless." An average based, as many now are, on data contributed by workers of world-wide distribution and over several

TABLE 43. — PHOSPHORUS CONTENTS OF A FEW TYPICAL FOODS

| ARTICLE OF FOOD      | NUMBER OF CASES | MEAN % P IN EDIBLE PORTION | PROBABLE ERROR (P.E.) OF MEAN | P.E. IN PERCENTAGE OF MEAN | COEFFICIENT OF VARIATION |
|----------------------|-----------------|----------------------------|-------------------------------|----------------------------|--------------------------|
| Milk . .             | 214             | 0.093                      | ± 0.0003                      | 0.3                        | 7                        |
| Eggs . .             | 104             | .224                       | ± 0.0014                      | 0.6                        | 10                       |
| Lean beef .          | 40              | .204                       | ± 0.0025                      | 1.2                        | 11                       |
| Almonds .            | 27              | .475                       | ± 0.0141                      | 3.0                        | 23                       |
| Dry beans .          | 32              | .463                       | ± 0.0066                      | 1.4                        | 12                       |
| Wheat . .            | 70              | .374                       | ± 0.0043                      | 1.1                        | 14                       |
| Sweet corn .         | 18              | .120                       | ± 0.0018                      | 1.5                        | 9                        |
| String beans         | 37              | .044                       | ± 0.0013                      | 3.0                        | 26                       |
| Apples . .           | 82              | .011                       | ± 0.00017                     | 1.5                        | 21                       |
| Dates . .            | 16              | .060                       | ± 0.0021*                     | 3.5                        | 21                       |
| Cabbage .            | 60              | .028                       | ± 0.0008                      | 2.9                        | 32                       |
| Kale . .             | 22              | .067                       | ± 0.0024                      | 3.6                        | 25                       |
| Asparagus .          | 16              | .052                       | ± 0.0032                      | 6.1                        | 35                       |
| Potatoes .           | 49              | .053                       | ± 0.0016                      | 3.0                        | 31                       |
| Beets . .            | 35              | .039                       | ± 0.0014                      | 3.6                        | 32                       |
| Carrots . .          | 47              | .040                       | ± 0.00125                     | 3.1                        | 32                       |
| Parsnips .           | 11              | .080                       | ± 0.0020                      | 2.5                        | 12                       |
| Turnips <sup>a</sup> | 155             | .032                       | ± 0.0006                      | 1.9                        | 36                       |

\* Includes 137 cases from the Rhode Island Agricultural Experiment Station representing very unusually wide variations of soil, fertilization, and cultural conditions.

years, may have a broader and more permanent validity than work done at any one time and place. For a given sample may be less truly representative, or a given analysis may be less accurate, than the individual investigator supposes. Nor are the analyses of today necessarily more accurate than those of a generation ago; for the subject-matter of chemistry has grown so greatly that it is much harder now than it was then to find time for rigorous training in quantitative analysis. And many of the new methods represent

gains in speed or convenience but not in accuracy, sometimes at the cost of accuracy.

**Relative nutritional availability of the calcium of different foods.**

As this section must be very short, it may conveniently take the form of a brief summary of three series of experiments made respectively by Rose, by Rose and MacLeod, and by Fincke.

Rose (1920) studied the utilization of the calcium of carrots in calcium-balance experiments with healthy young women to whom she fed diets carefully planned to furnish just about the maintenance requirement of calcium, one half of which was in the form of carrots. Under these conditions the calcium of the carrots was nearly as well utilized as that of milk.

Rose and MacLeod (1923) made a similar study of the utilization of the calcium of almonds. When almonds furnished about 73 per cent of the total food calcium, the calcium of the almonds was almost as well utilized as the calcium of milk or of carrots; but when the almonds were made to furnish about 85 per cent of the total food calcium the efficiency of utilization of the calcium of the almonds was somewhat less.

Fincke's experiments were made with animals, so that the retention of calcium could be studied over a much longer segment of the life-cycle, and could be determined by the direct analysis of the body at the end of the experimental period. Rats were chosen because their omnivorous food habits and the chemistry of their nutrition are in most respects so closely like those of human beings. The experimental animals were fed, through a period during which normal growth and development involves a relatively large increase in the calcium content of the body, on carefully planned diets in which calcium was the limiting factor and in which half of the calcium was furnished alternatively by the different foods to be compared. It was found that the calcium of milk was well utilized, that of kale almost as well, while the calcium of spinach was utilized to only a very slight extent, if at all (Fincke and Sherman, 1935).

From this and other similar investigations (Fincke and Garrison; Kao, Conner, and Sherman; Kung, Yeh, and Adolph; Mallon, Johnson, and Darby; Speirs; Tisdall and Drake), it now appears clearly that the calcium of celery cabbage, Chinese cabbage, collards, kale, leeks, lettuce, rutabaga leaves, tendergreen, and turnip greens is well utilized in nutrition; while that of spinach and New Zealand spinach is almost if not entirely useless, probably because

of the oxalic acid contained in these (and doubtless other) leaves of plants of the Goosefoot Family (Chenopodiaceae).

As the same general methods that have been used to compare the availability of the calcium of leaves will doubtless be applied to other foods, it should be emphasized that such experiments must be made in rather large numbers and in a strictly side-by-side manner before one is justified in attempting quantitative comparison of the findings.

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## CHAPTER XV

### IRON AND COPPER IN FOOD AND NUTRITION

#### Importance in Normal Nutrition

THE AMOUNT of iron contained in the body is small — only about one part in 25,000 of the body weight or about a tenth of an ounce in a full-grown healthy person — yet its functions are prominent and fundamentally important. It is an essential element of the oxygen-carrying hemoglobin of the blood, of muscle hemoglobin, of the chromatin substances which in some sense preside over the most vital activities of the cell, and of other substances whose functions, especially in connection with oxidation-reduction (redox) systems, are only beginning to be worked out.

Widespread and continuous as are the functions of iron compounds in the tissues, yet the greater part of the iron which the body contains is normally concentrated in the blood. If we accept the estimates that, in round numbers, the blood constitutes about 7 per cent of the body weight and contains about 70 per cent of the body iron, then obviously the concentration of iron is about ten times as high in the blood as in the body as a whole; and thirty times as high in the blood as in the average of the other parts of the body. And very much the largest part of the iron in the blood is present as an essential constituent of the hemoglobin of the red blood cells. Hence an iron-poor body tends to be anemic; and, while anemias may be due to many other causes as well as lack of iron, the studies of the adequacy of iron supply in nutrition have been closely interwoven with the studies in anemia, of hemoglobin formation and regeneration, of the history and functioning of the red cells or corpuscles of the blood.

More recently it has been found that copper is in some way involved in the process of hemoglobin formation. While its action is catalytic in the sense that it does not appear as a constituent of the hemoglobin formed, yet this action of copper in hemoglobin formation and regeneration \* is now regarded not as a drug effect

\* To a considerable extent, some of the parts of a catabolized hemoglobin mole-

but as part of the normal nutritional process. Hence copper is now counted among the nutritionally essential elements; and the study of iron and copper in nutrition, of the nutritional anemias and hemoglobin formation and regeneration, are so closely interdependent as to be scientifically inseparable.

### Blood Formation and a Classification of Anemias

**Formation of red corpuscles.** It is generally taught that in the normal adult, red cells are formed only in the bone marrow. After an average life of about six weeks, they are destroyed, either by fragmentation in the blood stream or by phagocytosis by endothelial cells, especially in the spleen. As the corpuscles are broken down, the hemoglobin is split into an iron compound and bilirubin. The bilirubin is carried to the liver by the blood stream and there secreted in the bile.

A large part of the products of breakdown of hemoglobin, both of the iron-containing and of the bilirubin fractions, is retained in the body and re-used in hemoglobin formation.

The number of red cells and percentage of hemoglobin is slightly higher in men than in women, and these also vary somewhat with age. The newborn child has about 40 per cent more red cells and hemoglobin per unit volume of blood than has the adult. During the first three months of life there is usually a decrease to about 65 per cent of the adult values. Between the ages of three and six months this usually rises to about 75 per cent of the adult values, after which there is continued gradual increase until the adult condition is established.

As contrasted with a normal condition of the blood, the term anemia is generally applied either to a deficiency of hemoglobin, or of red corpuscles, or both.

The relationship between the hemoglobin and the number of red cells (corpuscles) is important in classifying various types of anemia. It is expressed either directly as *mean corpuscular hemoglobin* (usually in millionths of a milligram) or as the *color index*.

$$\text{Color index} = \frac{\text{Hemoglobin (\% of normal)}}{\text{Number of red cells (\% of normal)}}$$

culc are normally used over again in making new hemoglobin; there is no sharp distinction between hemoglobin formation and regeneration.

**Recent research on anemia, considered from the nutritional point of view.** As an outgrowth of studies upon the significance of substances secreted in the bile, Whipple and his coworkers were led to an extended investigation of the regeneration of hemoglobin after experimental hemorrhage, and the influence of different foods upon the rate of such regeneration in dogs maintained upon a standard basal diet. Liver was found to be effective in this connection. Minot and Murphy then tried liver clinically in a very different kind of anemic condition, namely, in pernicious anemia. Here also it proved effective, and this clinical finding has prompted a very interesting nutritional research, one of the results of which has been the finding in liver extract of a specific curative substance. Simultaneously, there has been active nutritional investigation of still another type of anemia, an anemia produced experimentally in laboratory animals by the long-continued feeding of iron- and copper-poor food, especially during the period of growth. To make clear the differences between the different types of anemia and to guard against confusion in their correlations with the normal metabolism of iron (and copper) there follows a present-day classification of the anemias with discussion of the nutritional considerations involved.

### *Classification and Nutritional Discussion of the Anemias*

Modern classifications of the anemias differ somewhat in form. Sometimes the basis of classification is the change in the blood picture, and sometimes the grouping is in accordance with the cause of the departure from the normal. Of the latter type is that of Haden (1935), on which the following is based:

- I. Increased blood loss
  - (a) Mechanical loss (hemorrhage): See below.
  - (b) Accelerated destruction in the body: as in certain types of infections and poisoning, and certain familial abnormalities.
- II. Depressed blood formation
  - (a) Depressed bone marrow function: due to poisons, infections, nephritis, tumors of the bone marrow.
  - (b) Deficiency of specific substances: See below.
    - (1) Deficiencies of substances involved in hemoglobin formation (hypochromic anemias).
    - (2) Pernicious anemia type.

From the point of view of strictly nutritional aspects the two outstanding types are therefore:

- I. Those due to loss of blood (hemorrhage).
- II. Those due to deficiency of some substance or substances essential to hemoglobin or red cell formation.

Among deficiencies we distinguish

- (a) those of substances which are concerned with the formation of hemoglobin — iron, copper, and certain organic factors.
- (b) those having rather to do with the formation and development of the red corpuscles — “the specific substance which is effective in curing pernicious anemia.”

**I. Anemias due to hemorrhage.** The first effect of a large loss of blood is to reduce the volume in circulation. The bulk is rapidly restored by the absorption of fluid from the tissues, which dilutes the hemoglobin and red corpuscles.

In the regeneration of the blood, new red cells are formed more rapidly than hemoglobin. The reserves of pigment-forming substances in the body are easily exhausted, so that while the stroma or framework of red corpuscles is restored, their hemoglobin content is deficient. In this state, the blood is said to have a low color index.

The result of chronic loss of small amounts of blood is quite similar.

Regeneration of the normal blood in these cases appears to be chiefly a problem of restoring the hemoglobin. Whipple has shown that this is markedly influenced by the diet. Both organic and mineral factors are important. The former are most abundantly supplied by liver and kidney, while pancreas, brain, apricots, and peaches are also effective and doubtless other foods may be found so when investigated. Of the salts, iron compounds are most effective, copper and zinc less so.

Although these experiments led to the use of liver in pernicious anemia, it is now believed that the action in the two conditions is different and involves different factors, one favoring the formation of hemoglobin (after loss of blood) and the other the red corpuscle stroma (recovery from pernicious anemia). Extracts

rich in the factor active in pernicious anemia are much less potent in the anemia of hemorrhage.

In human beings, on ordinary diets, iron is practically always effective in promoting recovery from the anemia of hemorrhage, and the few reports upon use of liver have shown little if any effect. In the treatment of clinical anemia, ferrous salts seem more effective than ferric, and the amounts of iron thus used therapeutically are much larger than could feasibly be supplied by the food.

On the other hand, Miller and Rhoads (1934) have shown that it is possible, by bleeding followed by injection of hemoglobin, to bring about an anemia in which the chief need is for substances involved in the building of the red cell stroma as distinguished from its hemoglobin. While this is not the exact analogue of any recognized clinical condition, it indicates that hemorrhage may deplete the body of some substance or substances needed in other aspects of blood formation than the production of hemoglobin. The material here needed may perhaps be the substance specifically effective in pernicious anemia.

**II. Anemias due to deficiency of some substance or substances.** Here the deficiency may be the fault of the food or of digestion or absorption.

*Deficiency of hemoglobin-forming substances.* The number of red cells may or may not be reduced, but characteristically there is a low color index.

The relatively long-continued feeding of exclusive milk diet to rabbits or rats results in an anemia which seems to be due to deficiency of both iron and copper. If the milk accidentally contains enough copper, this experimental milk anemia can be cured by giving iron; but otherwise both iron and copper are needed, the iron as an essential constituent of the hemoglobin, and the copper to promote the process of hemoglobin formation.

This method of experimentation has been much used as a means of testing the availability of the iron and copper contents of foods, or the *hematopoietic properties* of food regardless of whether iron, copper, or some unknown factor is the limiting factor in the particular case.

It is still an open question to what extent such experiments bear upon the human problem. From the standpoint of the relative life cycles, it takes a month in the life of a child to be equivalent to a day in the life of a rat; and so it would be necessary to confine

a child absolutely to an exclusive milk diet for years in succession in order to parallel the experimental nutritional anemia induced as just mentioned in rats.

Moreover, it has been found that the above-mentioned decrease of hemoglobin which takes place in the first three to six months of human life is physiological rather than pathological and is not prevented by giving iron (apparently, either with or without copper); and the blood does not show the low color index or other characteristics of nutritional anemia. The consensus of medical opinion is that only a very small proportion of the cases of anemia which occur among infants below six months of age can be attributed to shortage of iron, or copper, or both.

During the last months of intrauterine life relatively large amounts of iron and copper are stored in the liver of the fetus; so that the body at birth is relatively rich in both of these elements. Correspondingly, milk shows relatively lower iron and copper content; but as the iron (and presumably the copper) of milk is readily assimilated and economically used in the body, the fact that the infant (born iron-rich) is chiefly nourished with milk for several months does not involve any serious danger of a nutritional shortage of iron. As insurance against any slight danger of such shortage, the foods introduced early into the diet of the child are chosen with reference partly to their iron content, as well as largely with reference to their vitamin values. (Dietaries adequate in iron and vitamin values are almost sure to contain ample amounts of copper and manganese.)

Recent research also indicates that a considerable part of the iron which disappears from the *circulation* of the infant during the physiological decrease of hemoglobin content is retained in the body stores available for resynthesis into hemoglobin with the growth of the child.

Around the age of six months, the child needs an adequate supply of iron to maintain the increased hemoglobin formation, and if, as often occurs in premature infants, twins, or the offspring of anemic mothers, the body stores are low, the iron content of the food then becomes a matter of importance.

Chlorosis, an anemia of young women, probably also belongs in this group, although the studies of iron metabolism here are not conclusive. This condition was very common forty to fifty years ago, but is rare now. It is associated with poor hygiene,



constipation, and malnutrition, with iron probably the chief deficiency.

*Idiopathic hypochromic anemia* is the term now applied to an anemia characterized by a low color index and not associated with hemorrhage or any other known cause. It occurs most frequently in women about thirty to fifty years of age and is usually associated with a gastric anacidity. In many cases the diet has been shown to be of low iron content, and it is thought that failure to absorb the iron (because of the defect in the gastric juice) may be equally important in bringing about a relative shortage of iron in the body (Mettier, Kellogg, and Rinehardt, 1933). The losses of iron in menstruation may also be a factor. Liberal intakes of iron produce rapid improvement.

*Pernicious anemia group.* The nature of the deficiency here is quite different. The substance specifically effective in pernicious anemia has to do with the production and development of the body or stroma of the red corpuscle rather than with its hemoglobin.

This type of anemia is characterized chiefly by deficient formation of red cells, which are greatly reduced in numbers and vary in size and shape. There is a relative abundance of hemoglobin, with a high color index, large stores in the muscles and excess of iron deposited in the liver, spleen, and kidneys.

A characteristic feature of pernicious anemia is deficient gastric secretion.

Castle has shown that the necessary factor is produced in the digestion of beef by normal gastric juice. Neither beef nor normal gastric juice alone had any effect on pernicious anemia, but an incubated mixture of the two had the same action as liver.

Since this type of anemia occurs in patients with deficient gastric secretion, he concluded that the normal person is able to produce the protective substance by the action of the *intrinsic factor*, present in the gastric juice, upon the *extrinsic factor* furnished by the food. Individuals with pernicious anemia lacked the intrinsic factor and were therefore unable to form the essential protective substance. This hematopoietic material \* was, however, supplied preformed by the feeding of liver or kidney. The feeding of stomach is also effective, whether because of containing the

\* The term *hematopoietic* is or may be somewhat loosely applied to whatever results in blood regeneration in a given case. Hence according to circumstances it may be applied to the combination of the intrinsic and extrinsic factors, or to either of them, or even to some other substance needed for hemoglobin formation.

same hematopoietic material as liver, or because it supplies the intrinsic factor. After absorption the active substance is stored in the liver, which explains the relative richness of liver as a source of this material.

Liver extract injected intramuscularly is much more effective than when given by mouth.

The extrinsic factor of Castle has now been shown (Miller and Pritchard, 1937) to be present in milk, egg, beef muscle, wheat germ, and autolyzed yeast. In coagulated milk this factor remains with the curd.

The purified preparations of the liver substance effective in pernicious anemia have relatively little effect upon the regeneration of hemoglobin after hemorrhage. The effect in pernicious anemia is, as suggested above, chiefly upon the formation and maturing of the red cells rather than upon their hemoglobin.

Among other types belonging to the pernicious anemia group are: Cases observed in India in which the deficiency is that of the extrinsic factor; cases of lack of intrinsic factor through destructive disease or surgical removal of the stomach; sprue and certain other diseases of the intestines, in which the active material apparently is not absorbed in adequate amounts.

It may happen that the same patient has both the hemoglobin deficiency and the pernicious type of anemia.

Anemia of pregnancy may be of either of the main types described above, or a combination of them. During pregnancy there is likely to be deficiency of gastric secretion at the same time with withdrawal of body iron for the fetus, thus increasing the danger of both the hypochromic and the pernicious type of anemia. The storage of iron in the fetus appears to depend to a large extent upon the amount in the mother's diet, and the completeness of its absorption.

From the foregoing, which attempts to summarize the medical classification of the anemias with only brief running comment upon nutritional relationships, it will be seen that the three lines of research which have recently attracted much attention from students of nutrition, namely, the work upon the anemia resulting from hemorrhage, that upon anemia induced by exclusive milk feeding, and that upon pernicious anemia, really deal with different conditions.

The condition studied by Whipple and his coworkers is that

which follows the withdrawal of blood from an otherwise normal and well-nourished body. It is to be kept in mind that what the body has to replace in such experiments is not any one substance, merely, but whole blood. Under the conditions of these experiments it is found that regeneration of hemoglobin tends to be the dominant feature in the situation. This is probably due at least in large part to the use in these experiments of a basal ration which provides for other nutritional needs while throwing upon the particular food (or drug) under investigation the special function of providing the material needed for hemoglobin formation.

In striking contrast with the condition existing in the experimental anemia of hemorrhage, is the condition found in "true" or typical pernicious anemia, where there is no lack of hemoglobin as such (and presumably no lack of the substances which enter into its synthesis in the body), but rather a lack of something involved in the production of the stroma — the framework of the red corpuscle which holds the hemoglobin and carries it throughout the body. The substance wanting in this type of anemia probably does not enter into the hemoglobin molecule at all! But it is a highly specific substance essential to the (process of) formation of red blood cells \* without which the hemoglobin itself cannot function effectively in the service of the body as a whole. Plainly the situation here is so far different from that of hemorrhage that we must be careful to avoid the confusion which is apt to arise from the fact that these two conditions are both called anemia.

A third type of anemia recently much studied is that induced experimentally by feeding young animals *exclusively* upon milk for an abnormally long time. The resulting condition is different from either of the two which have just been discussed.

It is important to keep in mind that the three kinds of anemia which have recently been so actively and successfully investigated are really three quite different states.

Whipple has said that a monument erected to commemorate developments in iron metabolism would be, indeed, a Tower of

\* A frequent feature in the blood of pernicious anemia is the decreased proportion of *young* red blood cells which are called reticulocytes in contradistinction to the fully developed erythrocytes. The rapid appearance of reticulocytes in the blood following the feeding of liver, or a potent liver extract, is an even earlier evidence of the success of the treatment than is the increase in the total red cell count, and, therefore, reticulocytes figure largely in the literature of this subject.

Babel, and that the confusion is largely due "to an attempt to apply findings in one type of anemia to a totally different type of anemia."

In the practical problem of the adequacy of the food supply to meet the nutritional needs of the body, the iron content of the food is doubtless a more important consideration than its copper content because the environment as a whole, with its accidental additions of copper to our food and drink, will almost always furnish us at least as much copper as we need.

It was also the opinion of the conference on treatment of diseases of the blood, held at the Cornell University Medical College and reported in the Journal of the American Medical Association in July 1940, that in only a small proportion of clinical anemias is there advantage in the addition of copper to the iron prescription.

### Quantitative Requirement for Iron in Nutrition

Comparatively few experiments upon the amount of food iron required for the maintenance of equilibrium in man have been made.

**Iron requirements of normal adults.** Farrar and Goldhamer (1935), basing their judgment both upon new experimental work of their own and upon a reexamination of the literature, reach a materially lower estimate of the iron requirement of the normal human adult than that given in previous editions of this book. In part, but only in part, this results from their construing "normal" in such a way as to exclude certain data which the writer deems it safer to include in the drawing of an average for general use. The experimental evidence which now seems directly applicable to the question, How much iron suffices for the daily needs of normal men and women?, may now be summarized as follows:

Data on iron requirement of normal adult human nutrition (to 1935):

|   | MGM. PER DAY |
|---|--------------|
| Lehmann (Cetti) . . . . .                 | 7.3          |
| Lehmann (Breithaupt) . . . . .            | 7.7          |
| Stockman and Greig { (1st case) . . . . . | 6.3          |
| (2d case) . . . . .                       | 3.7          |
| (3d case) . . . . .                       | 8.9          |
| (4th case) . . . . .                      | 11.5 *       |
| Von Wendt { (1st case) . . . . .          | 11.0         |
| (2d case) . . . . .                       | 9.0          |

|  | MGM. PER DAY                       |
|--|------------------------------------|
| Sherman { (1st case) . . . . .                               | 5.5                                |
| (2d case) . . . . .  | 8.7                                |
| (3d case) . . . . .  | 12.6 *                             |
| Reznikoff <i>et al.</i> . . . . .                            | 10.0 *                             |
| Ohlson and Daum . . . . .                                    | 14.9 *                             |
| Farrar and Goldhamer { (1st case) . . . . .                  | 5.2                                |
| (2d case) . . . . .  | 7.1                                |
| (3d case) . . . . .  | 7.8                                |
| (4th case) . . . . .   | 9.1                                |
| Vahlteich, Funnell, MacLeod, and Rose { (1st case) . . . . . | 6.2                                |
| (2d case) . . . . .  | 5.7                                |
| (3d case) . . . . .  | 6.1                                |
| (4th case) . . . . .   | 6.1                                |
| Average of all the above . . . . .                           | 8.11; P. E. $\pm$ 0.39; C. V. 32.9 |
| Average omitting those marked * . . . . .                    | 7.14; P. E. $\pm$ 0.29; C. V. 24.6 |

Here are only 21 cases as against about 100 cases each in the corresponding study of calcium and phosphorus requirements. With so few, and such variable, data, the average must be regarded as highly tentative. Pending further investigation, it may perhaps be well to think in terms of an average indication of about 8 milligrams of iron per day as needed for normal men and women (the lower body weights of women being offset by the blood loss in menstruation, so that men and women are regarded as needing equal amounts of iron).

If to this estimate of about 8 milligrams we add the same 50 per cent "factor of safety" as is customary in similar considerations of protein, calcium, and phosphorus, we arrive at a *general allowance* or "dietary standard" of 12 milligrams of iron per day instead of the 15 milligrams which has been much used as a standard allowance hitherto.

This would mean that in planning dietaries and in judging the nutritional adequacy of food supplies, the anxiety hitherto felt regarding iron would be very considerably ameliorated; while a still larger measure of relief will result if one is governed by Farrar and Goldhamer's still lower estimate of the normal iron requirement.

*Notes on some individual experiments.* Cetti and Breithaupt eliminated 0.0073 and 0.0077 gram of iron per day, respectively, when fasting. Three men observed by Stockman while receiving in the food about 0.006 gram each per day eliminated 0.0063, 0.0089, and 0.0115 gram, respectively. Von Wendt found his

requirements to range, in a number of experiments on different diets, from 0.008 to 0.016 gram per day, the largest amount being required in a case where the diet was deficient in calcium. Farrar and Goldhamer's data are for three men and one woman living under relatively constant dietary and other conditions. In three experiments by Sherman, in which the food contained 0.0057 to 0.0071 gram of iron, there was metabolized 0.0055, 0.0087, and 0.0126 gram per day, respectively, and the amount of iron which sufficed for equilibrium when taken in the form of bread and milk was insufficient when taken in the form of a diet consisting of bread and egg white, or bread alone. The nitrogen, phosphorus, calcium, and iron balances for two of these experiments performed upon the same man, and with diets practically alike in energy value and protein content, are shown in Table 44.

TABLE 44. — COMPARISON OF BALANCES OF DIFFERENT ELEMENTS

| NATURE OF DIET        | ELEMENT    | AMOUNT IN GRAMS PER DAY |          |          |          |
|-----------------------|------------|-------------------------|----------|----------|----------|
|                       |            | In Food                 | In Feces | In Urine | Balance  |
| Bread and milk . .    | Nitrogen   | 10.10                   | 0.46     | 13.09    | - 3.45   |
| Bread and egg white . | Nitrogen   | 10.69                   | 0.75     | 13.21    | - 3.27   |
| Bread and milk . .    | Phosphorus | 1.55                    | 0.57     | 1.03     | - 0.05   |
| Bread and egg white . | Phosphorus | 0.38                    | 0.22     | 0.75     | - 0.59   |
| Bread and milk . .    | Calcium    | 1.89                    | 1.34     | 0.15     | + 0.40   |
| Bread and egg white . | Calcium    | 0.10                    | 0.34     | 0.07     | - 0.31   |
| Bread and milk . .    | Iron       | 0.0057                  | 0.0053   | 0.0002   | + 0.0002 |
| Bread and egg white . | Iron       | 0.0065                  | 0.0085   | 0.0002   | - 0.0022 |

Here, although the nitrogen balance was practically alike on the two diets, there was on the bread and milk diet practical equilibrium of phosphorus and iron and a storage of calcium, while on the diet of bread and egg white there were noteworthy losses of all three of these elements.

Ohlson and Daum's observations upon young women "under essentially unrestricted conditions of living and diet" should probably be regarded as studies of the fluctuations of iron metabolism which occur under *ordinary* conditions, rather than as attempts to measure the *minimum* intake which would suffice. That is, these experiments were designed for other purposes than

the determination of the actual *requirement*. Hence in order not to overweight their data in a use somewhat foreign to its real purpose, the present writer includes in the above tentative compilation only the figure for average output which Ohlson and Daum include in their summary.

There is need of further study of the actual (normal minimum) iron requirement; but in planning such studies it should be kept in mind (a) that a satisfactory degree of accuracy in the operations requires extraordinarily meticulous technique, and (b) that however accurate the experimental work its interpretation is inevitably doubtful unless the intake is *controlled* at a uniform *low* level.

Preliminary report of an investigation of this kind by Leverton (1940) indicates the actual requirements of the four college women studied to be between 4 and 7 milligrams of iron (as furnished by ordinary foods) per person per day. That Barer and Fowler (1940) interpret their findings as indicating a higher need may be due to the fact that they worked with patients many of whose diseases very probably increased the wastage of iron beyond the normal rate.

**Iron requirement in pregnancy.** The fact that nature provides the baby with a special body store of iron before its birth necessarily means a somewhat increased need for iron by the mother during the gestation period. "Dietary standards" may take account of this and set a higher allowance of food-iron for pregnant women than for other adults. Strauss of the Harvard Medical School recommends a daily allowance of iron salt as a part of the routine dietary of prospective mothers. This makes a more generous provision for iron intake than could feasibly be made by food selection, and has the further advantage that with an abundance of iron provided as salt the choice of food can be made with reference to other nutritional considerations.

**Iron requirements of children.** In the experiments of Rose and coworkers (1930), a girl 31 months of age and weighing 31 pounds (13.95 kilograms) received a diet which for the purpose of this experiment was made poorer in iron by the omission of the egg yolk which it had regularly included. With the intake of iron thus reduced to 4.64 milligrams per day, the average output was found to be 5.74 milligrams per day, indicating that something more than the latter amount ought to be provided by the food. From the practical point of view, what most readily suggests itself is

the restoration, to the diet of the child, of the egg yolk which had here been omitted for the purpose of the experiment.

The experimental findings of Leichsenring and Flor (1932) were considerably lower, namely, 1.47 to 2.48 milligrams per day for maintenance in children with body weights of 15 to 19 kilograms. They follow Rose, however, in suggesting a much higher figure as a practical dietary allowance. Daniels and Wright (1934) and Ascham (1935) also favor a very liberal allowance. For new evidence see Porter (1941.)

**To what extent is iron requirement influenced by the nature of the diet?** In the writer's opinion the best answer to this question is that it cannot be answered yet; and that recent attempts to answer it are so likely to be misleading that one should maintain a high degree of "scientific skepticism" in this field. Great caution should be exercised in the acceptance and dietary application of estimated differences in the availability of the iron in different foods as tested by dipyrldyl or any other *in vitro* reagent or by feeding in any form of experimental anemia. The experimental production of anemia sets up a condition so highly special and so artificial that it is very doubtful which of the comparisons thus made should, and which should not, be regarded as having direct application to normal nutrition and dietetics.

When sufficiently developed, the evidence of balance experiments upon normal people and the quantitative comparisons of laboratory animals fed upon different diets all within the range of normal but some more nearly optimal than others, will probably afford much sounder guidance.

Also, there are reasons for believing that either a deficient or a grossly excessive intake of calcium may unfavorably influence the assimilation of iron.

### Iron in Foods

Table 45 shows the approximate amounts of iron now believed to be present in the average edible portion of typical food materials expressed (1) in milligrams per 100 grams of edible material, (2) in milligrams per 100 grams of protein, (3) in milligrams per 3000 Calories.

Percentages of iron in many other foods will be found in the Appendix. Using data for iron in food materials which were earlier than the present average figures but in essential agreement with



them, approximate estimates of the amounts of iron contained in over 300 American dietaries have been made. The majority of these were found to furnish 15 to 22 milligrams of iron per man per day. Apparently therefore the typical American dietary does not contain such a large surplus of iron as would justify leaving the supply of this element entirely to chance; but does contain enough so that iron is not so often a danger-point as is calcium.

TABLE 45. — IRON IN TYPICAL FOOD MATERIALS

| FOOD                     | IRON PER 100 GRAMS<br>FRESH SUBSTANCE | IRON PER 100<br>GRAMS PROTEIN | IRON PER 3000<br>CALORIES |
|--------------------------|---------------------------------------|-------------------------------|---------------------------|
|                          | <i>milligrams</i>                     | <i>milligrams</i>             | <i>milligrams</i>         |
| Beef, all lean . . . .   | 3.0 <sup>a</sup>                      | 13                            | 80 <sup>a</sup>           |
| Beefsteak, medium fat    | 2.0 <sup>a</sup>                      | 13                            | 43 <sup>a</sup>           |
| Eggs . . . . .           | 3.1                                   | 24                            | 60                        |
| Egg yolk . . . . .       | 8.7                                   | 53                            | 73                        |
| Milk, whole . . . . .    | .2                                    | 6                             | 9                         |
| Oatmeal . . . . .        | 5.2                                   | 37                            | 39                        |
| Rice, polished . . . .   | .7                                    | 9                             | 6                         |
| White flour . . . . .    | 1.3                                   | 12                            | 11                        |
| Wheat, entire grain . .  | 5.7                                   | 51                            | 48                        |
| Beans, dried . . . . .   | 10.3                                  | 47                            | 90                        |
| Beans, string, fresh . . | 1.1                                   | 46                            | 78                        |
| Beets . . . . .          | .9                                    | 56                            | 59                        |
| Cabbage (average) . . .  | .7                                    | 50                            | 74                        |
| Carrots . . . . .        | .7                                    | 58                            | 47                        |
| Kale . . . . .           | 2.5                                   | 64                            | 151                       |
| Peas, dried . . . . .    | 6.0                                   | 25                            | 52                        |
| Potatoes . . . . .       | 1.1                                   | 55                            | 39                        |
| Spinach . . . . .        | 3.4                                   | 148                           | 413                       |
| Turnips . . . . .        | .5                                    | 45                            | 43                        |
| Apples . . . . .         | .3                                    | 100                           | 14                        |
| Bananas . . . . .        | .6                                    | 50                            | 18                        |
| Oranges . . . . .        | .3                                    | 33                            | 18                        |
| Prunes, dried . . . . .  | 3.5                                   | 152                           | 35                        |
| Tomatoes . . . . .       | .6                                    | 60                            | 79                        |

<sup>a</sup> Figures for meats can be only rough approximations because of variations in fatness, as well as differences between different cuts. Forbes and Swift report that organs contain more iron than muscle meats, while pork and lamb contain much less than beef.

In fact, the very gratifying increase in the per capita consumption of fruit and vegetables during recent years (plainly shown by the market statistics of the United States Bureau of Agricultural Economics, and illustrated also by the recent studies of Gillett and Rice in New York City) must mean that the present average iron

content of American dietaries is somewhat above that indicated by the data cited in the preceding paragraph. Apparently, the combined effect of the teaching of the newer knowledge of nutrition and the development of methods of preservation and transportation, marketing facilities, and market news services, has already been sufficient so that, while the importance of iron in food and nutrition should still be carefully taught, the practical importance of "stress" in the teaching is not so great in the case of iron as it is in the cases of calcium and vitamins A and C.

### Copper Content of Foods

Lindow, Elvehjem, and Peterson (1929) published results of determinations of copper in a wide variety of foods. Their summary of these data is (in part) as follows: The figures range from 44.1 milligrams of copper per kilo of fresh calf liver to 0.1 milligram of copper per kilo of fresh celery. Within these limits the various groups of foodstuffs in order of their average copper content per kilo of fresh material come as follows: ten kinds of nuts, 11.6 milligrams; four of dried legumes, 9.0 milligrams; nineteen of cereals, 4.7 milligrams; eight of dried fruits, 4.2 milligrams; four kinds of poultry, 3.0 milligrams; seventeen kinds of fish, 2.5 milligrams; thirteen of animal tissues, 1.7 milligrams; two of green legumes, 1.7 milligrams; eleven of roots, tubers, stalks, and bulbs, 1.4 milligrams; fourteen of leafy vegetables, 1.2 milligrams; twenty-seven of fresh fruits, 1.0 milligrams; ten of non-leafy vegetables, 0.7 milligrams. The authors pointed out that the first four groups owe their high copper contents largely to the low percentages of moisture contained in these foods.

Since that time many determinations of copper in foods have been published by other investigators.

Averages, which take account of all available data for the foods included, may be found in Appendix C.

### Characteristics of Some Types of Food as Sources of Iron and Copper

*Meats.* In ordinary muscle meats the iron exists chiefly as hemoglobin, belonging in part to the muscle cells and in part to retained blood. Since fatty tissue contains much less iron, the iron content of fat meat is much lower than that of lean, and in order to establish a useful general estimate of the amount of iron in

meat it seems best to refer the iron to the protein content rather than to the gross weight of the meat. The results will still be influenced by the extent to which the blood has been either accidentally or intentionally removed from the muscle.

For fresh lean beef (containing the usual proportion of blood), the results collected by the writer averaged 0.00375 per cent iron, but Forbes and Swift (1926) reported considerably lower results, 0.0024 to 0.0025 per cent. Hence, in computing the data for Table 45, a value intermediate between the averages of the two sets of findings has been used.

Some years ago, chiefly as a means of avoiding the serious discrepancies which might otherwise arise from the great variability of meat in fatness, the writer suggested that a rough estimate of the amount of iron furnished by the meat of a dietary might be made by assuming that with every 100 grams of protein the meat would furnish about 0.015 gram (15 milligrams) of iron. This estimate Forbes and Swift consider to be "a little high for beef and veal, and much too high for lamb and pork, while it does not apply at all closely in relation to heart, brain, liver, spleen, kidney, and blood." All of these latter are such minor products in comparison with ordinary muscle meats that even if completely utilized as human food their effect would be to raise but slightly the percentage of iron in the meat supply as a whole. Hence it appears from the work of Forbes and Swift that the custom of assuming in dietary calculations that meats furnish about 15 milligrams of iron per 100 grams of protein has somewhat overestimated the value of beef and veal, and much overestimated that of lamb and pork, as sources of iron; but that the use of this factor becomes more nearly correct as products such as liver, spleen, kidney, and blood are being more largely utilized as human food. It should, however, always be kept in mind that any such single factor can serve merely for the discussion of meats as a whole and not for the comparison of one meat with another.

The copper content of beefsteak was found by Lindow, Elvehjem, and Peterson to be about 1 part in 1,000,000; and other beef products showed very similar figures, except liver which contained, weight for weight, about 20 times as much copper as muscle tissue.

*Eggs.* The edible portion of hens' eggs has shown as the average of several analyses 0.00303 per cent of iron. Whether the iron content of eggs can be increased by giving to poultry food rich in

iron, is a disputed question. It seems probable that both the relatively high iron content of the egg and its copper content of about two parts per million are properties rather definitely fixed by nature.

There can be no doubt regarding the assimilation and utilization of the iron compounds of eggs, since they serve for the production of all the iron-containing substances of the blood and tissues of the chick, there being no introduction of iron from without during incubation.

*Milk.* Analyses of samples of cows' milk of widely different origin have given results varying both with the milk and with the analyst, and averaging about 0.0002 per cent of iron in the fresh substance.

The iron of milk is readily absorbed and assimilated. Moreover, metabolism experiments indicate that the iron of milk is likely to be utilized to especially good advantage, perhaps on account of its association with other materials of high "body building" value.

The Wisconsin investigators find that milk contains only 0.000015 per cent of copper; and that neither the iron nor the copper content of milk is much influenced by the feeding of iron and/or copper salts as additions to the normal ration of the cow. The mineral content of milk is apparently rather well stabilized by nature. The fact that the Wisconsin workers find a higher copper content in calves' than in beef liver may perhaps be taken as an indication that the body of the young mammal is thus provided with a reserve store of copper as well as of iron at birth.

*Grain products.* Iron occurs in considerable quantity in the cereal grains, but the greater part of it is in the germ and outer layers, and so is rejected in the making of the "finer" mill products, such as patent flour, polished rice, and new-process corn meal. In view of the part which the iron of the germ takes in the sprouting of the seed and the nutrition of the young plant, there is little room for doubt that it is of value also in the animal economy. To test the value of the iron in the outer layers of the grain Bunge carried out the following experiment:

A litter of eight rats was divided into two groups of four each. One group was fed upon bread from fine flour, the other upon bread made from flour including the bran. At the end of the fifth, sixth, eighth, and ninth weeks, respectively, one rat of each group was killed, and the gain in weight, the total amount of hemoglobin,

and the percentage of hemoglobin in the entire body were determined.

Here the bran-fed rats not only made a much greater general growth, but developed both a greater amount and a higher percentage of hemoglobin. There can be no doubt that the iron and other ash constituents of the outer layers of the wheat were well utilized in these cases.

The recent work of M. S. Rose also shows clearly the good utilization of the iron of whole wheat in hemoglobin formation. Ascham, Spiers, and Maddox (1939) found the iron of black-eyed peas, green and yellow split peas, navy beans, pinto beans, and butter beans "completely available" for hemoglobin formation.

The fruits and fresh vegetables are often regarded as of low nutritive value because of their high water content and low proportions of protein and fat. But it is largely this property which makes them especially important as sources of food iron, because they can be added to the diet without replacing the staple foods of high calorific and protein value, and without making the total food consumption excessive. Present transportation facilities and methods of drying and preserving tend to equalize the cost and increase the available variety of fruits and vegetables throughout the year.

In an experimental dietary study made in New York City it was found that a free use of vegetables, whole wheat bread, and the cheaper sorts of fruits, with milk but without meat, resulted in a gain of 30 per cent in the iron content of the diet, while the protein, fuel value, and cost remained practically the same as in the ordinary mixed diet obtained under the same market conditions. This was found by the use of the data available some years ago. Peterson and his coworkers suggested that fruits and vegetables generally may have even higher iron values than hitherto appreciated. This led to a large number of new determinations of iron in fruits and vegetables by Stiebeling, who also computed new general averages which are here used in the revised values given in Table 45 above and in the more comprehensive tables in the Appendix. In many cases these general averages are the results of analyses of so many samples that they are not likely to be much changed by future work; but on the whole the figures for iron are still subject to relatively larger probable error than are the corresponding data for calcium and phosphorus.

### Summary and Conclusion

A combination of circumstances has resulted certainly in much confusion regarding "the iron problem" in nutrition, and probably in the assumption by students of food and nutrition of an excessive feeling of anxiety and responsibility regarding this problem or these problems. A few facts should therefore be emphasized at this point in order that the reader may conclude this chapter with a sound sense of proportion.

The frequency of occurrence of anemia does *not* imply a corresponding frequency of iron deficiency in the food supply. Even such anemias as are curable by iron are not to be assumed to have been due to iron-poor food. The therapeutically effective doses are so many times larger than the amounts of iron contained in normal dietaries that the anemia cured by iron clearly may involve something more than could have been prevented by dietary planning.

Recent iron-balance studies show the normal nutritional requirement for iron to be somewhat less than previously estimated. Thus the "dietary standards," current for something like a generation past, provide a more generous margin above actual average need than we supposed. This reassuring fact is not to be offset by either of two doubts which seem to be causing undue anxiety at present: (1) Doubts of the availability of the iron of ordinary foods seem to be greatly exaggerated by the currency given to certain findings obtained by methods of investigation of so artificial a nature as probably to have little bearing upon human problems; but in any case it is to be remembered that it is by means of iron balance experiments with people subsisting on ordinary food that our estimates of iron requirement are arrived at, so that our finding of an average requirement of say 12 milligrams means 12 milligrams *as contained in ordinary foods*. This still remains true if one believes that only three-fourths of the iron of ordinary foods is "available" in the sense of some conventional use of this word. (2) Another source of undue anxiety is an apparent tendency to overestimate the iron requirements of normal women. Iron-balance experiments, the real significance of which was to *survey prevailing levels and fluctuations* have sometimes shown negative balances which have been erroneously supposed (by some) to indicate the need of a higher intake. The fact, long recognized as fundamental by critical investigators but apparently not fully appreciated by

all readers or even all writers, is that, *only when the intake is sufficiently low and sufficiently uniform* can the balance be trusted to afford any valid evidence as to requirement. When the intake is not thus controlled, the data of the balance are very apt to give a seriously exaggerated impression as to requirement. Another source of exaggerated impressions of the average needs of women for dietary iron is the fact that the menstrual losses of some women are abnormally high. If such cases are included in an average, the average thus becomes higher than that of *normal* need. Such ambiguities have doubtless tended to exaggerate some recent estimates of the iron requirements of women. It would be much more logical to recognize the existence of the idiopathic cases and see that they get medicinal iron under medical advice, than to try to get "idiopathically high" amounts of iron into dietaries generally through an exaggerated emphasis upon iron. For most of the foods of especially high iron content are either rather fibrous or rather expensive. Rather than distort the dietary, or run the risk of too much roughage, or skimp other parts of the food budget in order to include large amounts of iron-rich foods, it is probably much wiser to be satisfied with moderate margins of food-iron, keeping in mind the fact that any *special* iron requirement may be met by iron salts on the same principle that iodized salt is used to meet iodine requirement as explained in the chapter which follows.

With iron as with protein, deficiency conditions are encountered in the body which are not due to faulty food but to some aberration on the part of the body itself. Even if such conditions are improvable by nutritional means, still the problems which they present are essentially medical, and the responsibility of management of such cases belongs to the profession of medicine rather than to the chemistry of normal nutrition.

When the student of normal nutrition is forewarned against misleading complexities of this "Tower of Babel era" in the study of iron metabolism and anemia, and is careful not to encroach upon problems which belong to medicine, it may appear that notwithstanding a somewhat confusing situation our recent knowledge is on the whole rather reassuring as to the margin of safety above normal need carried by the iron contents of the food supplies of most communities.

The present-day student of nutrition is entitled to the enjoyment of such moments of reassurance when they are encountered,

for our study of several other factors will necessarily leave us with the impression that new knowledge has increased the burden of nutritional responsibility.

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## CHAPTER XVI

### IODINE IN NUTRITION: SIMPLE GOITER AS A NUTRITIONAL PROBLEM

#### Natural Distribution of Iodine and Its Relation to Goiter

IODINE is one of the essential chemical elements of the human body, although it constitutes only about one part in three million parts of the body weight. Sea salt, and therefore the spray which evaporates in the air at the seashore, is relatively rich in iodine; and as this sea-salt dust is carried inland by the wind it gives iodine to the rain-water, the soil water, the soils, and the crops of nearby regions. Also, some rocks contain enough iodine to yield significant amounts of soluble iodide in their natural weathering. But regions whose water supplies do not come from such rocks, and which, because of too great distance or because of intervening mountains, receive practically none of the air-borne sea-spray, may contain in their waters and in the crops grown on their soils too little iodine to meet the needs of normal nutrition. The people who grow up in such regions show a high proportion of simple goiter, which is primarily an enlargement of the thyroid gland resulting from its being obliged to function without an adequate iodine supply. Of course, these glands may also become infected. When the ordinary foods and drinking waters of a region are too poor in this element, the small amount of iodine needed to prevent goiter can be supplied by adding iodide to the drinking water or the table salt; by incorporating some iodine compound into tablets with food or confectionery; by the use of sea foods; or by consuming in or with one's food a small amount of any of various sea plants not hitherto used as food, which have been found to be rich in iodine.

The recent tendency in discussions of the variations in iodine contents of waters and foods has been to lay more emphasis upon the chemical nature of the rocks in the mountains, and less upon the spray of the sea, than was customary in previous years. There



may even be a significant difference in this respect between the rocks on the two sides of the same mountain range.

The regional variations of incidence of goiter were recognized both in Europe and in Asia before its relation to iodine deficiency was understood.

As a recent striking example of an iodine-poor region, McCollum and Simmonds (1929, p. 395) cite Keith's study of the Pemberton Valley in the Pacific Northwest. There, many of the men, most of the women, and almost all the children had enlarged thyroids. The domestic animals also showed great prevalence of goiter. As a result of providing iodine, either as iodized salt or as sea food, most of the trouble has now been rectified.

It should be borne in mind that, while lack of iodine is often, and probably usually, the dominant factor in producing simple goiter, infections arising from contaminated water supplies or generally unhygienic conditions may also play an important part, as has been particularly emphasized by McCarrison. In a more recent discussion of goiter, however, McCarrison has emphasized the fact that the more nearly perfect the nutritional intake the more nearly perfect will be the thyroid gland, both as to its size and structure and as to its functioning.

McClendon and Williams (1923) correlated the iodine content of drinking water with the prevalence of goiter in four zones in the United States as follows: With 0.01–0.1 part of iodine per billion of water the goiter rate was 15–30 cases per 1000 of population; with 0.015–1.2 parts per billion of iodine, 5–15 cases; with 0.06–9.0 parts per billion, 1–5 cases; with 1.4–10 parts per billion the rate was less than 1 case per 1000. They also produced enlarged thyroids by deprivation of iodine in controlled laboratory experiments upon rats.

McClendon, Barrett, and Canniff (1934) have furnished another illustration. They find the average iodine contents of Minnesota potatoes to be: 85 parts per billion for the eastern, and 226 for the western section of the State. Correspondingly the records show an incidence of 19 per thousand of simple goiter among men drafted for the World War in the eastern and only 7.5 per thousand in the western section.

The same lack of iodine which causes goiter may also cause myxedema which is attributed to an insufficiency of thyroxine, a nutritionally active iodine compound formed in the thyroid gland.

### Iodine and the Thyroid Gland: Thyroxine

For an excellent and non-technical introduction to the glands of internal secretion the reader is referred to Hoskins' *The Tides of Life* (1933).

In 1895, Baumann discovered iodine in the thyroid gland. Subsequent work showed that this iodine is intimately connected with the activity of the thyroid. The characteristic physiological effects of administration of thyroid gland appear to be directly proportional to the iodine content of the gland administered.

In 1914, Kendall isolated from thyroid a pure crystalline substance containing about sixty-five per cent of iodine, which substance he named thyroxine and showed to be capable of exerting the characteristic effects of thyroid upon metabolism.

In 1926, Harington determined the structure of thyroxine to be as shown in Chapter V. Very interesting in this connection is the isolation by Foster (1929) of 3, 5 di-iodotyrosine from thyroid.

Whether thyroxine circulates and functions in the body in its free state or in combination with protein, as in thyroglobulin, or in both ways, is still a subject of discussion. Heidelberger and Pedersen (1935), considering thyroglobulin to be the principal protein elaborated in the thyroid gland and, "as appears probable, . . . the actual thyroid hormone," have studied it by modern physicochemical methods and find evidence that its molecular weight is of the order of 675,000 or around twenty times as large as that of insulin. As it is difficult to picture the direct diffusion of such large protein molecules through cell membranes these investigators suggest that the thyroglobulin may be deposited on the surface of a cell, and then either exert its action by means of its loosely bound thyroxine groups, or be hydrolyzed by the proteolytic enzymes of the cell with a gradual liberation of free thyroxine at the surface of the cell whose activity it catalyzes. Such a concept helps us to understand the relatively slow and long action of the thyroid hormone as compared with most of the other hormones which have so far been investigated.

Kendall (1929) and Harington (1933) give full accounts of the laboratory work of isolating and of synthesizing thyroxine and many interesting illustrations of the use of the products thus obtained in the clinical treatment of diseases which had resulted from the inadequacy of the thyroid secretion (goiter, myxedema,

cretinism). The thyroid utilizes simple iodide in the preparation of thyroxine. Normally the small amounts of iodides contained in food and drink furnish sufficient iodine both for the proper functioning of the thyroid gland and the production by it of the hormone needed to regulate metabolism throughout the body.

When the amounts of iodine furnished by the food and drink are insufficient, the thyroid gland becomes enlarged. It is now generally agreed that this is the cause of most simple goiter, and that simple goiter is, therefore, in its origin an iodine-deficiency disease.

Seaweeds and sea water have long been the familiar sources of iodine. In regions where the people live largely upon sea food, and the atmosphere and drinking water are constantly receiving the iodine-containing salt spray blown in from the sea, the intake of iodine is presumably adequate and in such regions goiter is very rare; but in regions too remote or too mountainous to receive significant amounts either directly or indirectly from the sea, goiter is much more common, as in the Great Lakes region and much of the Northwest of the United States, in parts of Switzerland, and in several other parts of the world.

Marine and Kimball, reasoning that if the prevalence of goiter in such regions is due to a lack of iodine it should be preventable by giving iodide to children at the age at which goiter ordinarily begins to develop, obtained permission to try their now classical experiment in the public schools of Akron, Ohio. Here as many as volunteered of the pupils of the ages known to be most susceptible to goiter were given small doses of sodium iodide dissolved in drinking water twice weekly over a period of a month and repeated twice yearly. As is well known, this experiment was strikingly successful. In only 5 cases among over 2000 treated was there any enlargement of the thyroid gland when iodide was taken, while in a similar number of children of the same age in the same region not taking iodide about 500 showed enlargement during the same time. Hence it would appear that 99 per cent of the simple goiter of this region could be prevented by iodide, if means for its universal administration could be found. This work of Marine and Kimball, first published in 1917 and subsequently (1921) confirmed and extended, has focussed attention upon the relation of iodine supply to goiter both in this country and abroad. In three Swiss cantons where an attempt was made to give iodide to all school

children during the three years 1918-1921, the incidence of goiter was diminished during the three years from 87 per cent to 13 per cent.

The general subject of the functioning of the thyroid gland is reviewed anew by Marine (1935) who emphasizes the importance of the interrelations of the thyroid with other organs both of internal and of external secretion, but adds that as to our knowledge in this field only a good beginning has been made. According to Marine, the fact that the principal function of the thyroid is to increase oxidative processes in the body indicates that all bodily activities are influenced by the state of functioning of the thyroid gland and *vice versa*. He estimates the normal iodine content of the (20 to 25 gram) human thyroid at an average of 10 to 15 mgms., with a maximum of 20 to 25 mgms. The thyroid gland retains iodine even during fetal life, the amount acquired depending largely upon the iodine intake of the mother.

Marine considers (1935) that the substance actually secreted by the thyroid is probably iodothyroglobulin (commonly called thyroglobulin) but that thyroxine appears to be the active part of the thyroglobulin molecule, natural and synthetic thyroxine both having the same effect as natural thyroid secretion, and showing the maximum degree of the effect somewhat more quickly.

Marine suggests that the means by which thyroxine increases oxidative processes in the cells is unknown; but that there is considerable evidence that epinephrine (adrenine, adrenaline) and thyroxine may work together in this process. There are also reasons for believing that thyroxine is always present and acting, though in differing degrees according to the amount secreted by the gland, whereas the throwing of epinephrine into the circulation by the adrenal glands may be essentially an emergency response. In this case, the two would act together in times of markedly accelerated oxidation rate, while thyroxine alone may suffice to maintain oxidation at such rates as obtain in times of relative quiescence. Marine also cites observations which suggest that diets rich in proteins and fats increase the rate of discharge of thyroxine and that "thyroid activity is more necessary in the oxidation of fats and proteins than of carbohydrates." In this same review, Marine (1935) gives considerable attention to the interrelations between the activity of the thyroid and the activities of other glands.

### Iodine Requirement of the Body

Evidently the body has a more or less definite nutritive requirement for iodine. Qualitatively this requirement might be stated as a sufficient amount of iodine to meet the daily losses from the body and maintain within the body such store as is needed to provide for the manufacture within the thyroid gland and the distribution throughout the body of sufficient amounts of thyroxine (thyroid hormone) to support a normal rate of physiological activity.

According to Marine, if the iodine content of the thyroid is maintained above 0.1 per cent of *its solid matter*, goiter does not develop. In the thyroids of healthy soldiers killed by war wounds Zunz found an average of about 0.05 per cent iodine *in the fresh substance*, which, as the glands weighed about 26 to 30 grams, amounts to about 15 milligrams of iodine in the thyroid gland of a full grown healthy man. According to the estimates of Kendall and Plummer the rest of the body may be expected to contain about 10 milligrams more of iodine, probably chiefly in the form of thyroxine which (whether free or in combination with protein) has been distributed by the thyroid and is serving to control metabolism in all the active tissues of the body.

Thus the full grown healthy man is estimated to contain in his 70 kilograms of body a total of about 25 milligrams of iodine, ~~equivalent to~~ part in about 2,800,000 parts of body substance or less than 0.00004 of one per cent of the body weight.

The difficulty of quantitative determination of iodine in the extremely minute amounts in which it occurs in most plant and animal tissues must be expected to render somewhat slow and uncertain the working out of satisfactory data on the iodine values of foods and the quantitative requirements of the body for iodine at different ages and under different conditions. Von Fellenberg estimates that the normal human adult requires about 0.000014 gram of iodine daily and that, when larger amounts are furnished by the food and drink, an easily mobilized reserve store of iodine is built up in the body. It is doubtless through the building up of such a body store of iodine that the administration of iodide for two weeks to one month twice yearly by Marine and Kimball proved so effective in preventing the development of goiter throughout the year as above noted. Adolph and Whang (1932)

estimated the per capita iodine intake in the non-goitrous coastal region of East China at 0.000032 to 0.000066 gram per day (32 to 66 $\gamma$ ). As yet there cannot be said to be a consensus of expert opinion as to the amount of iodine required in normal nutrition. Two of the reasons that this has not yet been established probably are: (1) that the reserve which the body tends to carry can be so conserved that negative balances do not soon become perceptible; and (2) that the actual amounts of iodine involved in human nutrition and contained in ordinary foods are so small that the "personal equation" in the use of even the best available analytical methods becomes a relatively large factor, and therefore, different investigators working upon the same absolute amounts may nevertheless reach quite different quantitative findings.

The prevention of goiter in regions whose soils and waters are very poor in iodine is a nutritional problem whose immediate solution may depend not so much upon detailed knowledge of iodine contents of foods as upon the use of methods which lie largely in the province of the health officer.

The success of Marine and Kimball in preventing the development of goiter through the giving to the school child of a little iodide in drinking water, naturally suggested the addition of iodide to public water supplies, and this has been done in several communities.

Two other methods have also received favorable attention: the administration of iodine compounds in chocolate tablets or some equivalent palatable form and the addition of a small proportion of sodium iodide to table salt intended for household use.

Olin (1924) described the state-wide survey of thyroid enlargement and the preventive measures undertaken in Michigan in part as follows:

"The survey showed to us the extent of our problem and it suggested the state-wide remedy. The unvarying relationship between the percentage of thyroid enlargement and the iodine content of the water supplies gave added proof that simple goiter was a nutritional rather than a medical problem. Making good the iodine deficiency in the state's food supply would solve the problem. The question was how to do this.

"Treatment of water supplies was promptly ruled out. This method has been tried and found not entirely effective in several cities. Added to that, it would affect only the cities at best, and our problem was as much rural as urban.

"Chocolate iodine tablets are already in use in the school systems of several cities of Michigan. Their effectiveness is beyond question, but again, this way of solving the problem applies best to cities with well

organized schools and continued educational propaganda among parents. It has the added disadvantage of failing to reach two important groups — the expectant mother and the preschool child.

"It was clear to us that a condition that was the result of a state-wide food deficiency could best be remedied by supplying that deficiency through an inexpensive and universally used food stuff. Salt was, of course, the logical medium to be chosen, since crude salt commonly contains iodine. Representatives of all Michigan salt manufacturers were therefore invited to Lansing and at an enthusiastic conference the agreement was reached whereby all companies would put on the Michigan market, on May 1, 1924, a new 'iodized' table salt."

Turrentine of the United States Department of Agriculture has emphasized the possibility that it may prove advantageous to give iodine in a form less readily soluble and less rapidly absorbed into the circulation than is a simple iodide solution, and has advocated the use of kelp and particularly preparations of the species *Macrocystis pyrifera* which he finds to be particularly rich in iodine; and Adolph and Whang (1932) find the *Laminaria religiosum* from the sea of the Chinese coast to be also a rich source of iodine.

We have here discussed the relation of iodine supply to the occurrence and prevention of simple goiter. It is not the function of this book to discuss the treatment of already developed goiters. Wherever the disease exists it should be under medical care. In adenomatous goiter, for instance, there are medical reasons for not giving added iodide; and partly for this reason there has recently been a tendency to reduce the proportion of iodide recommended for addition to the table salt for general sale in goitrous regions.

The use of salt containing one part of sodium (or potassium) iodide to from 5000 to 200,000 parts of sodium chloride, as a *preventive*, is simply a matter of normal nutrition. The added iodide is not to be regarded as a drug but rather as restoring the table salt to something like its natural composition — the ordinary table salt of today being the product of a highly artificial refining process and being thus in a sense denatured. Thus in the case of Charleston, W. Va., cited by McCollum and Simmonds (1929, p. 401), the community up to 1898-1900 had used relatively natural salt from local salt wells and had been practically free from goiter; but between 1898 and 1900 this was almost entirely displaced by the now familiar refined table salt and thereafter a larger proportion of the school girls began to show enlarged thyroids.

Kimball (1928), after years of practical experience as a physician giving special attention to goiter, stated explicitly that there is no real danger in the use of iodized salt for the prevention of goiter and emphasized the importance of such prevention both in childhood and in pregnancy.

More recently, the American Medical Association's Committee on Foods has repeatedly approved iodized salt for general use, and in at least one announcement it is noted that the brand of salt under consideration in that particular case contained also a little sodium carbonate to stabilize the added iodide. There seems now to be a consensus of scientific opinion that iodized salt can be made a satisfactory means of supplying the nutritional need for iodine. Obviously, then, to the extent that iodized salt is utilized, the iodine content of foods becomes a matter of abstract scientific interest rather than of practical dietetic anxiety. Yet so long as there remain regions in which the iodine content of the drinking water is low and the use of iodized salt is not practically universal, cases will occur in which the adequacy of the iodine intake may depend upon the choice and the source of the food.

### Iodine Content of Foods

In the case of iron or copper the great difficulty of quantitative studies of nutritive requirement lies in the extreme smallness of the amounts concerned in the body's daily intake and output. In the case of iodine this difficulty is increased many-fold. As a matter of fact, the quantities of iodine in most foods are so small that the analytical methods used in examining foods for iodine have not usually been very conclusive even as to whether iodine is present or absent. Doubtless in many cases iodine has been reported absent in foods which really contained it, but in quantity too small to be found by the analytical method employed. It is sometimes stated or implied that ordinary foods do not furnish iodine and that unless sea foods are used the iodine intake will depend entirely upon the drinking water; but this is too pessimistic a view. McClendon has analyzed a number of foods from a number of regions and has seldom if ever reported the absence of this element from any natural plant or animal product.

Nevertheless the data in Table 46, compiled from the work of several investigators, will serve to illustrate (1) the wide variation of iodine content in foods, (2) the divergence of findings of



different workers upon the same food, and (3) the tendency of foods from goitrous regions to show less iodine than food of the same sort from non-goitrous regions. Note that the figures are only *milligrams of iodine per metric ton of dry matter in the food, or parts per billion*.

TABLE 46. — IODINE IN FOODS FROM GOITROUS AND NON-GOITROUS REGIONS: PARTS<sup>†</sup> PER BILLION OF DRY MATTER

| KIND OF FOOD            | GOITROUS REGIONS | NON-GOITROUS REGIONS | AUTHORITY                              |
|-------------------------|------------------|----------------------|--|
| Wheat . . . . .         | 1-6              | 4-9                  | McClendon                              |
| Oats . . . . .          | 10               | 23-175               | "                                      |
| Carrots . . . . .       | 2                | 170                  | "                                      |
| " . . . . .             | —                | 507                  | Okla. Agr. Expt. Sta.                  |
| Lettuce . . . . .       | —                | 618                  | " " " "                                |
| Potatoes . . . . .      | 85               | 226                  | McClendon                              |
| " . . . . .             | —                | 10-216               | Frear                                  |
| " . . . . .             | —                | 90-700               | Georgia Agr. Expt. Sta.                |
| Cabbage . . . . .       | —                | 776                  | Adolph and Whang                       |
| Cranberries . . . . .   | —                | 26-35                | Morse                                  |
| Asparagus . . . . .     | —                | 946                  | Okla. Agr. Expt. Sta.                  |
| Radishes . . . . .      | —                | 994                  | " " " "                                |
| Tomatoes . . . . .      | —                | 379                  | " " " "                                |
| Milk . . . . .          | 265-322          | 572                  | Remington and Supplee                  |
| Butter . . . . .        | 140              | —                    | McClendon                              |
| <i>Sea foods:</i>       |                  |                      |  |
| Codfish . . . . .       | —                | 5,350                | U. S. Bureau of Fisheries <sup>a</sup> |
| Conch . . . . .         | —                | 1,140                | " " " " "                              |
| Crabmeat . . . . .      | —                | 1,460                | " " " " "                              |
| Flounder . . . . .      | —                | 1,480                | " " " " "                              |
| Oysters . . . . .       | —                | 1,800-3,500          | " " " " "                              |
| Red Snapper . . . . .   | —                | 1,440                | " " " " "                              |
| Salmon . . . . .        | —                | 570-2,200            | " " " " "                              |
| Shrimp . . . . .        | —                | 1,100                | " " " " "                              |
| Cod liver oil . . . . . | —                | 7,670                | " " " " "                              |
| " " " . . . . .         | —                | 3,000-13,000         | Holmes and Remington                   |

<sup>a</sup> Coulson, 1935.

Fellenberg also found more iodine in the food of the non-goitrous regions of Switzerland than in the food of the Swiss regions where goiter is prevalent. In general Fellenberg finds the iodine content of grains and legume seeds to range from 8 to 64 parts per billion; of fruits from 6 to 120; of nuts up to 200; of vegetable

oils 30 to 95; of cod liver oil about 5000 parts per billion. Chile saltpeter contained in a fresh sample 192,000 and in an old sample 49,000 parts — such large quantities as to have some effect upon the iodine content of the soils and ground waters where this nitrate is liberally used as fertilizer. Mineral waters examined by Fellenberg showed results ranging from 11 to 6000 parts of iodine per billion. Fellenberg and his associates have also discussed, much more fully than space permits here, the distribution and circulation of iodine in organic and inorganic nature; and more recently other investigators have published quantitative work of similar purport.

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## CHAPTER XVII

### ASCORBIC ACID (VITAMIN C)

#### Discovery and Chemical Identification

AROUND the time of Columbus' discovery of America, scurvy was one of the most prevalent diseases in Europe. In fact one writer then suggested that all other diseases might be considered as outgrowths of scurvy. The medical literature of the European countries does not show clearly how long they had suffered from this disease. It is known to have been one of the afflictions of the Crusaders.

When Vasco da Gama, near the end of the fifteenth century, made his voyage around the Cape of Good Hope, he reported the death by scurvy of nearly two thirds of his crew.

In 1535 Cartier, obliged to winter in Canada, lost a quarter of his men by scurvy, and nearly all the others were severely ill with it. From the natives they learned that decoctions of the twigs and leaves of certain trees served to cure and prevent the disease.

Early in the following century the English settlers of New England suffered much from scurvy, and learned to make definite provision against it by the use of fruits and their products or the products of germinating grain.

Captain Cook and his men escaped scurvy by making a regular practice of stocking their ship with the fresh fruits of all the shores that they visited, giving the same careful attention to this as to the replenishment of their drinking-water.

What the Europeans suffered from scurvy during their exploration and colonization of the New World was compensated for in two ways: they learned the antiscorbutic values of fresh vegetables and citrus fruits so that as early as 1720 Kramer wrote of these as the sure preventive; and they brought the potato from America to Europe where, as potato culture became common, scurvy became relatively rare.

It continued, however, to afflict sailors on long voyages, and Lind as surgeon of the *Salisbury*, finding himself obliged to treat

an epidemic of scurvy with only scanty supplies, decided to give the available oranges and lemons to two of his twelve patients while others received respectively, cider, cream of tartar, elixir of vitriol, or other drugs which had been recommended by different medical writers. The account of this experience which Lind published in 1757 had, therefore, much of the character of a report upon a controlled experiment. The men receiving the oranges and lemons were completely cured, those who had cider showed improvement, while the other patients gradually became worse.

In 1841, Budd explicitly ascribed the *antiscorbutic property* possessed by certain foods to a *definite substance* which, he wrote, "it is hardly too sanguine to state, will be discovered by organic

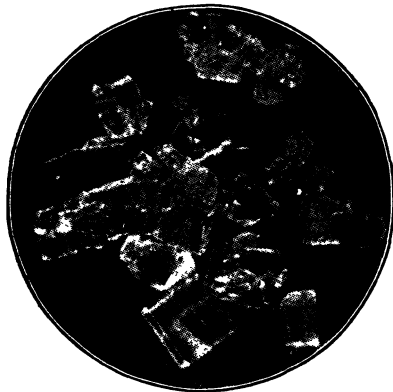


FIG. 28. — Crystals of ascorbic acid.  
(Courtesy of Dr. C. G. King.)

chemistry or the experiments of physiologists in a not far distant future." Ninety years later, Budd's prediction was fulfilled by C. G. King, employing the methods both of organic chemistry and experimental physiology in his researches at the University of Pittsburgh.

King's chemical identification of vitamin C, quickly confirmed by Szent-Gyorgy in 1932 and soon followed by the synthesis of the substance,

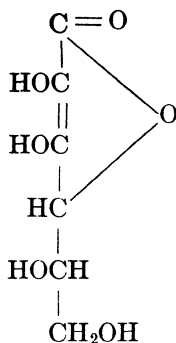
made this the first of the vitamins to be conclusively established as a substance of known molecular structure; as it may also be considered to have been the first to be clearly postulated as a definite chemical entity (by Budd, in 1841).

In the meantime, however, the vitamin concept had been independently developed through studies of normal nutrition and of beriberi, so that when the antiscorbutic substance was formally incorporated in the vitamin scheme of 1920 by Drummond, it was given only third place in the vitamin alphabet, McCollum having already designated a fat-soluble A and a water-soluble B.

To avoid monotony, we shall here make about equal use of the two names, vitamin C and ascorbic acid.

**Molecular Structure of Ascorbic Acid and its *in vitro* Determination**

Chemically, vitamin C is:



2, 3 dienol-*l*-gulonic acid lactone.

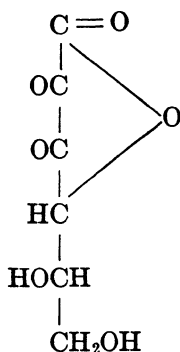
As no name indicative of this structure and of convenient length was forthcoming, the designation *ascorbic acid* has been generally accepted as being distinctive and historically appropriate.

It will be seen that in molecular structure ascorbic acid is closely related to the monosaccharide sugars, and to several of them, for of its carbon atoms only those in the 4 and 5 positions are asymmetric. This fact makes possible the synthesis of ascorbic acid from different carbohydrate starting-points. Thus the structure has repeatedly received independent confirmation. Also the pure synthetic substance is relatively inexpensive, and so is widely available as research material, as well as for therapeutic use.

Even before the substance had been isolated it was known to be readily oxidized. Its oxidation is much more rapid in alkaline than in acid solutions, and is markedly catalyzed by even traces of copper. Although ascorbic acid is, in general, the most labile of the known vitamins, and its destruction under ordinary conditions is chiefly due to oxidation, it is not autoxidizable within the normal pH range of plant and animal tissues. Rather, the rate of its oxidative destruction depends both upon its oxidation-reduction potential and a delicate interdependence of pH and the concentrations of other factors. (See Lyman, Schultze, and King, 1937, *J. Biol. Chem.* **118**, 757-764; and King, pages 328-330 of *The Vitamins*, 1939.)



As more fully summarized by King (l.c.), the oxidation-reduction potential of ascorbic acid is such that it is reversibly oxidized to dehydroascorbic acid



by a wide variety of organic compounds and of inorganic reagents.

Among the former are the indophenol dyes, and among the latter is iodine. Both these have been employed quantitatively for the determination of ascorbic acid, in many plant and animal tissues, including foods.

The method most generally adopted is that depending upon the carefully controlled oxidation of ascorbic acid to dehydroascorbic acid by the phenol-indophenol dye with the corresponding reduction of the latter to its leucobase, as developed by Bessey and King and currently modified for still greater accuracy. (Cf. Bessey, pages 354-364, *The Vitamins*, 1939.)

Because of the possible presence in urine of small amounts of some substance or substances of oxidation-reduction potential so similar to that of ascorbic acid as to involve the danger of being counted with it, Roe has developed a method for urine analysis in which ascorbic acid is determined not by oxidation but by means of a carbonyl condensation reaction. Even in the case of urine, however, the findings of the two methods do not seem to be so widely different as to involve great danger of seriously misleading results from the use of the phenol-indophenol titration.

### Destruction of Vitamin C by Catalyzed Oxidation

The conservation of the vitamin C values of foods depends upon protecting the vitamin from oxidation not only by the exclusion of air in the ordinary sense, but also by freeing the material from

dissolved air, the avoidance of copper and other such catalysts as might gain entrance from even brief contact with utensils as well as the longer contacts with containers, the maintenance of a low temperature, and of an acid reaction. It is also to be remembered that the oxidation hazards are not all from without; for if cabbage juice (to mention a single instance) is carefully acidified to the same pH as tomato juice, and the two are treated with exactly the same precautions in all the respects above mentioned, the vitamin C value will still fall more rapidly in the juice of the cabbage than in that of the tomato, because of the natural presence in the cabbage of substances of higher oxidation potential than in the tomato.

### **Numerical Expression of Vitamin C Values**

Vitamin C values and requirements are most satisfactorily expressed in terms of milligrams of the actual substance, and this plan is followed in the present book. The International Unit of vitamin C is now defined as 0.05 mg. of the actual substance.

### **Nutritional Chemistry of Ascorbic Acid**

Prominent as is the oxidation-reduction behavior of ascorbic acid, the exact way in which this property functions nutritionally in the animal body has not yet been fully and clearly worked out. It seems reasonable to look forward to the further elucidation of this chemical relationship in the near future (possibly by the time this is read).

Meanwhile the nutritional function of ascorbic acid which has thus far been most fully set forth is its relationship to the formation and maintenance of intercellular cement substance (or substances).

Wolbach (1937) has especially emphasized the view that vitamin C controls the ability of the tissues to produce intercellular material; and that failure or decline of this ability from shortage of vitamin C is the underlying cause: (1) of the hemorrhages which may occur anywhere in the body; (2) of profound changes in the structure of the teeth and gums; (3) of changes in the growing ends of the bone with beading and other deformities which formerly were mistaken for rickets; (4) of the falling apart of bones due to loss of supporting cartilage; (5) of enlargement of the heart and damage of the heart muscles; (6) of degeneration of muscle fibers generally, causing extreme weakness and even death; (7) of the anemia due to destruction of blood-forming cells in the bone marrow

and loss of blood by hemorrhage; (8) of loss of calcium through degeneration of the bone matrix, the bones sometimes becoming so soft that they break spontaneously; and (9) of a degeneration of the sex organs.

Obviously, if shortage of vitamin C may have any of such widely distributed results in the body, one's individual bodily constitution may determine how and where the penalty will be paid for neglect to carry ample nutritional insurance in the daily food intake of this important factor in the body's internal environment.

Thus vitamin C is much more than merely an antiscorbutic. When scurvy has become a thing of the past, vitamin C will doubtless still figure prominently in medical and nutritional problems.

King and Menten (1935) showed that vitamin C makes the body more able to withstand diphtheria toxin. Experimenting with guineapigs they found that this difference was manifest before there were any signs of scurvy among the animals on the lower level of vitamin C intake. These specific findings are given a broader significance by the establishment of a definite relation of this vitamin to immunological complement (Chu and Chow, 1938; Ecker and Pillemer, 1939).

From the further work of Ecker and Pillemer it may be computed that from 4 to 8 times as much vitamin C per day is needed (at least by the guineapig, and very likely also by man) to maintain an optimal complement-value of the blood as to prevent symptoms of scurvy. This condition of "full complementary activity" corresponded in their experiments with the maintenance of a concentration of 1.0 milligram of vitamin C per 100 cubic centimeters of guineapig blood serum. This is about the same concentration of vitamin C that most investigators of the subject have considered practicable and desirable to maintain in human blood. As investigators of the vitamin C concentration of the blood of apparently healthy human beings have found (*e.g.*, personal communication from Dr. F. L. MacLeod, August, 1940) it is at about this same level, — in some individuals a little below and in others a little above 1.0 "milligrams per cent," — that it begins to be difficult further to raise the concentration of this vitamin in the blood except by a seemingly disproportionate increase of the intake.

Much clinical work indicates that vitamin C requirement is specifically increased in "colds," tuberculosis, rheumatic fevers,

and probably several other diseases; as also in the normal processes of pregnancy and lactation.

It is probable that health can be built to higher levels, and that a more favorable foundation for specific therapy against tuberculosis, rheumatic fever, rheumatoid arthritis, and doubtless other diseases, can be supplied, in part, through intakes of vitamin C much more liberal than the amounts which suffice to prevent scurvy.

Gray has expressed the relation of vitamin C to the intercellular substance, and the significance of the latter to tissue structure, by likening this binding material to a pliable cement and its microscopic darker bands of firmer texture to the binding strips of steel embedded in reinforced concrete. When there is shortage of vitamin C these bands fail to form, the intercellular substance becomes more liquid, less binding, and cells are not so well secured in their precise positions. The frequent hemorrhages which accompany scurvy and certain forms of anemia may be consequences of this weakness in the intercellular substance, the cells forming the walls of the small blood vessels ceasing to hold compactly together, and the blood leaking between them.

The relation of vitamin C to the teeth deserves discriminating consideration.

Enthusiastic reports of a few years ago, attributing to this vitamin a specific value in the combat of dental caries, met considerable scientific skepticism. The evidence on this point is conflicting and the question is still debated. The inconclusive state of the caries problem, however, should be distinguished from some other aspects of "the tooth problem" on which the evidence is much clearer.

It had earlier been found \* that vitamin C is highly essential to the good internal structure of the teeth; and more recently Boyle, Bessey, and Wolbach † have definitely shown the value of this vitamin in the prevention of the systemic type of pyorrhea. Thus the soundness both of the teeth themselves and of their supporting bones and gums is importantly dependent upon the amount of vitamin C supplied by the food.

*Experimental vitamin C deficiency.* Before the chemical identification of vitamin C much quantitative work had already been

\* As described more fully in pages 162-167 of Sherman and Smith's *The Vitamins*, 2nd Ed. (1931).

† Whose paper is listed among Suggested Readings at the end of this chapter.

done in the study of its distribution and stability under various conditions, making use of *in vivo* methods ("bio-assays") for its determination, usually by the feeding of standardized guineapigs under controlled conditions with precise observation of the degree of scurvy thus induced.

While it is now but seldom deemed necessary to make use of the feeding method as a quantitative procedure, the experience which was gained in such determinations is very useful as a guide to the experimental production of graded degrees of scurvy for purposes of demonstration. The method used, and typical results obtained, in the Columbia laboratories may be outlined as follows:

Young guineapigs, 6 to 8 weeks of age and weighing 250 to 300 grams are placed individually in cages, or in groups in a small pen, and fed *ad libitum* a basal diet consisting of: Oats, sound whole grain ground in the laboratory as needed, or, a mixture of about equal parts commercial rolled oats and wheat bran, 59 per cent; skimmed milk powder heated on open trays at 110° C. until all antiscorbutic vitamin is destroyed, 30 per cent; butterfat, freshly prepared, 10 per cent; table salt, 1 per cent. (This diet may be reinforced with yeast if desired.) Water is given *ad libitum*.

With no antiscorbutic, there is usually good initial growth followed by onset of scurvy symptoms, cessation of growth and great loss of weight before death from scurvy, which occurs at 26 to 34 days. Autopsy reveals in severe form all the typical signs of scurvy, notably hemorrhages, fragility of bones, and looseness of teeth.

With 1.0 cc. of tomato juice \* per day, the duration of life is prolonged and becomes less uniform. The animals become lame and stiff before death, and at death show severe hemorrhages, fragile bones, and loose teeth.

With 1.5 cc. of tomato juice per day, the animals usually live out the experimental period of 70 to 90 days after which it is unlikely that death from scurvy will occur. Such animals develop scurvy symptoms, conspicuous sensitiveness and stiffness in the joints, and usually loss in body weight. Hemorrhages and enlargements of rib junctions may become quite as pronounced as in the previous cases. (Since the animals live longer there is more time for these abnormalities to develop.) Fragility of bones and looseness of teeth are less marked than when less antiscorbutic is given.

\* Either raw or canned tomatoes may be used as source of this juice; but the juice itself, having been exposed to air in its preparation, should not be stored long before

With 2.0 cc. of tomato juice per day, growth after 15 days is subnormal and the animals show soreness of joints without noticeable stiffness. When these animals are killed and examined after 70 to 90 days on this allowance they show hemorrhages but not to a pronounced degree.

With 3.0 cc. or more of tomato juice per day, there is complete protection from scurvy as judged by examinations both during life and at autopsy; and growth should be normal in all of those test animals that eat the basal diet well.

An integrated numerical expression can be given in the form of a *scurvy score* obtained by adding together on the score card principle the indications of scurvy found in a standardized form of autopsy record.

Another plan is to conduct experimental feedings essentially as described but to judge the degree of protection from examinations of the teeth alone.

### Concentration-Levels of Ascorbic Acid in the Body

The distribution of vitamin C in the body and the relation of levels of intake and output under different conditions have been much studied since the chemical identification of the substance and the development of rapid methods for its quantitative determination.

King and his coworkers carefully investigated the concentrations of the substance in the organs, tissues, and fluids of the body; while Harris *et al.* reported a marked dependence of urinary output both upon the current nutritional intake and upon bodily condition.

Harris and his coworkers found \* that, when other conditions are relatively constant, the urinary output of vitamin C tends to vary with the nutritional intake. The larger the amount of ascorbic acid received in the daily food, the higher the concentration-level of this vitamin in the blood, and the larger the amount passing into the urine.

That is, in the long run, higher levels of daily intake result *both* in higher levels of vitamin C in the body and higher levels of output through the kidneys.

\* See, for example, Abbasy *et al.* (1935, 1936, 1937) and Harris *et al.* (1936, 1937) in the list of references and suggested readings at the end of the chapter; and, as in all such cases, it is well to search the libraries available to you for articles (or abstracts of articles) published since the compilation of the lists in this book.

Moreover (still assuming uniformity of other conditions), the higher the level of vitamin C that the body already has in its tissues and fluids, the larger the percentage of any given extra intake (test dose) which will overflow from the blood into the urine. A high percentage recovery in the urine thus indicates that the body was already well stocked with vitamin C; whereas if it were being maintained on a low level of "vitamin C nutrition" it would add more of the test dose to its stores and excrete less through the kidneys.

Thus the quantitative study of recovery of vitamin C in the urine following the ingestion of a standard test dose becomes a means (not entirely conclusive by itself) of studying the "condition of vitamin C nutrition" in the body.

Moreover, such studies throw light not only upon the abundance or paucity of the body's stores or content of ascorbic acid, but also upon the rate at which this vitamin is being used-up. Workers of the Harris group usually obtained notably smooth and clear-cut results in their studies of the relation of output to intake; but also found that common infections (even a light cold) lowered the urinary output of ascorbic acid, presumably because of an increased rate of destruction of the vitamin in the body.

Similar evidence of increased destruction of vitamin C in the body during infections has been reported by several independent investigators and for several different diseases. More of the vitamin is then needed if serious shortage in the body is to be avoided.

King and his coworkers have further shown (as already briefly noted) that shortage of vitamin C lowers the body's resistance to bacterial toxins, and that this lowered resistance may become serious before there is any external evidence of scurvy lesions. They also found that injection of bacterial toxins caused rapid and marked depletion of vitamin C in the body.

The amount of vitamin C contained in the body, the rate at which it is destroyed, and the rate at which it leaves through the kidneys, are all subject to relatively wide variation under the conditions of ordinary life; and do not depend solely upon the level of intake, though they may be largely influenced by it.

The differences are at least partly individual. Long ago it was observed that in a large group of children of the same age and environment some need more vitamin C than others. From their writings it would appear that Peary needed more antiscorbutic in

the arctic than did Stefansson. When the latter argues from his observation of the Eskimos that vitamin C may be less important than nutritionists think, he may give too little weight to the probability that the Eskimos (and perhaps some of the Norwegians also) may be the result of a "natural selection" of people whose vitamin C requirement is lower than that of most of us.

Moreover, Hoygaard and Rasmussen offer evidence that the typical food supply of the Eskimos actually contains very considerably more vitamin C than has been supposed, largely because of the unnoticed consumption of marine algae, as well as the fact of their eating liver and other parts *immediately* upon the killing of animal, bird, or fish.

That the amount of vitamin C required to maintain a given concentration of this substance in the blood is larger for some people than for others is also being found in current studies of the status of nutrition of American college students.

Recent investigations and discussions of "levels of vitamin C nutrition" have been based largely upon determinations of this substance in the blood plasma, with the concentrations stated in "milligrams per cent" which in this case may, without appreciable error, mean either per 100 grams, or per 100 cubic centimeters or milliliters.

An interesting illustration from the work of Farmer and Abt is shown in Fig. 29, from which it will be seen: (1) that one week of feeding of a pint of orange juice daily in addition to the previously customary diet brought up into the normal range all of their subjects whose initial blood values were subnormal; and (2) that this more liberal daily allowance of vitamin C in the diet also appreciably increased the level of concentration of vitamin C in the blood plasma (and probably in the body generally) of those whose initial levels were already within the normal range.

That even in the case of so soluble, diffusible, and labile a substance as vitamin C the body habitually carries a higher concentration when it receives a higher intake is a fact of much importance to our growing appreciation of the influence of one's daily food habits upon one's internal environment.

This flexibility of the body's internal environment (as contrasted with its previously postulated *fixite*) is very important both theoretically and practically; but of course there are both practicable limits and individual differences.



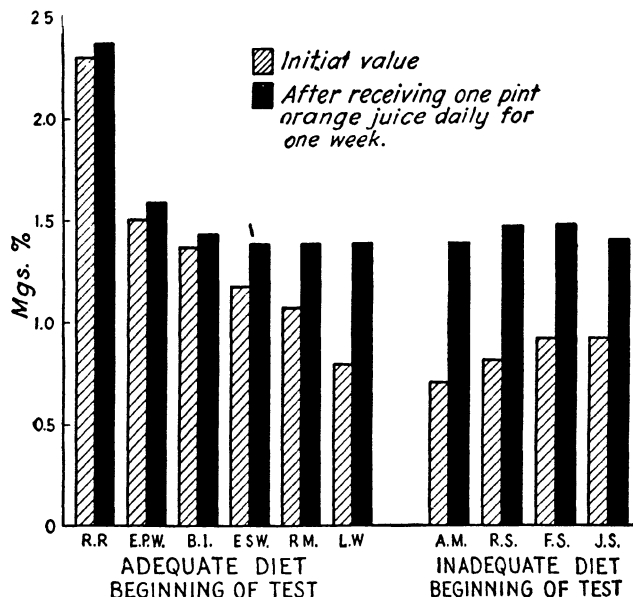


FIG. 29. — Vitamin C content of the blood as influenced by the diet. The six people on the left had all received apparently adequate diets prior to the beginning of the test. Note, however, the considerable individual differences among them, doubtless due mainly to their differing choices of food. The four people to the right, who were known to have had low dietary intakes of vitamin C all show low levels of vitamin C in the blood at the time of the initial determinations, results of which are indicated by the cross-hatched columns. After generous intakes of this vitamin for one week each person showed a gain, and those whose blood levels had been low are all now well up in the normal range. (From Farmer and Abt in *The Vitamins* by permission of the American Medical Association, publishers, and by courtesy of Dr. C. J. Farmer and the Milbank Memorial Fund.)

The interpretive comment of the *Journal of the American Medical Association* upon the recent clinical research of Minot and co-workers (1940) is that during the months in which fresh fruit and vegetables are scarce, about 60 per cent of the children (in a presumably representative American community) "had levels of ascorbic acid below the generally accepted satisfactory level of 0.7 mg. per hundred cubic centimeters of serum and about 30 or 40 per cent (of these children had) less than 0.3 mg." These investigators suggest that diets which are simultaneously deficient in calories and other essential factors may "suppress" the development of typical signs of scurvy in which cases even seriously

lowered levels of vitamin C concentration in the body may not be detected in ordinary physical examination.

What appears to be a carefully observed individual case of unusually high vitamin C requirement is reported by Rudolph (1940). A man receiving a "diet consisting of two kilograms daily of flesh, liver, heart, kidney, and marrow of antelope and an onion weighing 200 to 250 grams," the whole estimated to furnish 75 milligrams of vitamin C per day nevertheless developed scurvy within less than four months and was then cured by giving fresh fruit.

Another aspect of individual variability in vitamin C metabolism is illustrated in the very carefully controlled laboratory investigation of healthy human subjects by Todhunter and Fatzer (1940). Both before and after the addition of oranges to the dietary, their Subject A excreted more vitamin C in the urine while showing a slightly lower concentration of this vitamin in the blood, than their Subject B. The quantitative response in urinary output to a given increment of intake was about alike for the two subjects, indicating that they were about equally well "saturated," as would have been judged from the analyses of their blood but not from the analyses of the urines. Thus for a given status of bodily saturation, urinary output is higher in some individuals than others. Also, individual differences in the rate of urinary excretion of vitamin C after a test dose may be relatively large; so that the modification of method of studying urinary response which shortens the collection period to 6 hours instead of 24, introduces a considerably larger error with some subjects than with others.

Most people even when very liberally supplied with vitamin C do not readily maintain a plasma concentration above about 1.5 mg. per 100 ml. which is therefore commonly taken as about the upper limit of the *usual* normal zone.

It is more difficult to say what is or should be taken as the lower limit of normality. Some would set this boundary at 1.0 mg.; some at 0.7 mg.; some perhaps still lower, inasmuch as lower plasma concentrations may be found without scorbutic lesions.

Even before we had measurements of concentrations of ascorbic acid in blood, it was recognized that there may be deleterious results from shortage of the antiscorbutic factor before the appearance of scorbutic lesions. Thus Plimmer wrote that before definite symptoms of scurvy appear there is a period of poor health which may also occur in those who habitually take too little vitamin C,

though they get enough to prevent acute scurvy. Among the symptoms common in such cases are loss of energy and fleeting pains in the joints and limbs usually mistaken for rheumatism.

There is now much evidence that the absence of scurvy does not prove that the food is supplying all the vitamin C that the body needs for its best health and vigor.

Hess repeatedly pointed out how frequently children without showing any distinct scurvy symptoms are irritable, lacking in stamina, and more or less retarded in growth; and can be restored to better growth, higher stamina, and better general health and disposition by the feeding of additional vitamin C in the form of orange juice or other suitable antiscorbutic food, showing that the food supply had been too poor in vitamin C for the maintenance of really good health although no distinct symptoms of scurvy had appeared.

The work of numerous investigations seems to show that long-continued low intake of vitamin C increases the incidence of infectious disease, and conversely that the resisting power (or "positive" or "buoyant" health) of the body is increased by a diet containing liberal supplies of vitamin C.

In carefully controlled experiments of King and Menten (1935) in which guineapigs on graded levels of vitamin C intake received injections of graded amounts of diphtheria toxin, it was demonstrated, as briefly noted above, that the animals less liberally supplied with the vitamin, although they showed no external symptoms of scurvy, were much more sensitive to injury from the bacterial toxin at all dosages of the latter than were parallel animals on higher intakes of vitamin C.

Vitamin C probably functions in the nutritional processes of all species: those which do not need to have it in the food presumably make their own within their bodies.

Studies on the vitamin C content of animals tissues indicate that it is present in practically all tissues of the higher animals — in those of species (such as the rat and chicken) which thrive on diets containing no demonstrable amount of vitamin C, as well as in species (man, guineapig, and monkey) which are susceptible to scurvy. The *relative distribution in the body* is similar in both groups of animals, being highest in glandular tissue (in particular the adrenals, pituitary, corpus luteum, and young thymus) and lowest in blood and lean muscle; but the concentrations are higher in

those species which can synthesize vitamin C (and are therefore presumed to maintain tissue stores at or near optimal level) than in human subjects who have received an ordinary or conventional diet. In a series of analyses of human tissues obtained at autopsy, Yavorsky, Almaden, and King (1934) found wide individual variations and concluded that, in approximately one fifth of the people represented, the tissue concentrations of vitamin C were distinctly below what is conducive to optimal health.

Such facts bear upon the problem of the extent of the margin of safety which food supplies should provide.

### Problems of Adequate and Optimal Intakes

Scurvy, at least in its latent and preclinical or subclinical forms, is still of frequent occurrence and its prevention is a live problem even among populations ordinarily regarded as well fed. Hopkins has recently reported such an experience with the boys of a well-to-do English boarding school whose food conditions had been traditionally regarded as good, but where (as was found upon investigation) a fruit-shop formerly patronized by the boys was no longer available. In Canada, in Maine, in a high school of lower New York City, in Pennsylvania, and in the South, recent surveys have revealed very serious prevalence of low-grade scurvy or (in absence of recognized scorbutic lesions) such low levels of vitamin C nutrition as are now to be regarded as a hazard to health.

A daily intake of 25 milligrams of vitamin C by adults (other than women in pregnancy and lactation who need decidedly more) or 1.0 milligram per 100 Calories of food in family dietaries might be regarded as a minimum sufficing for the prevention of demonstrable deficiency.

In view of the facts now known, it would seem that anything to be called a standard allowance should provide a wide margin above the bare minimum as a safeguard for the body in its encounters with the varied everyday occurrences which may increase its rate of destruction, and therefore its level of need, of this vitamin.

Double the above amounts, or 50 milligrams for adult maintenance and 2 milligrams per 100 Calories for family dietaries, might be regarded as a *medium* standard.

By again doubling this latter, *i.e.*, by allowing 100 mg. per adult, or 4 mg. per 100 Calories of family food, one may approxi-

mate the *presumably optimal* allowance sufficing to keep the body "saturated."

The independent experimental investigations of Hauck, of Ralli, and of Todhunter, with their respective coworkers, agree in showing a daily need of 1.6 to 1.7 milligrams of vitamin C per kilogram of body weight, or about 100 milligrams for an average adult, to maintain the body in a condition of "vitamin C saturation."

Todhunter and Robbins<sup>1</sup> (1940) have further found that for maintenance of a blood plasma value above 1 mg. of vitamin C per 100 ml., 60 mg. per normal adult per day was required; while a daily intake of 120 mg. was needed to raise the concentration in the blood plasma to 1.4 mg. per 100 ml.

Space permits the mention here of only a few more of the recent publications bearing upon the problems of adequate and optimal intakes of ascorbic acid.

Goldsmith and Ellinger (1939) found a large proportion of patients to show on testing "a mild vitamin C deficiency" though showing no clinical symptoms. In "saturation" work they found that only when the vitamin C of the blood rose above 1.4 mg. per 100 cc. did urinary excretion increase materially, suggesting that this level ought to be maintained.

Lanman and Ingalls (1937) hold that the body store (degree of "saturation") of vitamin C is of more influence in the healing of surgical wounds in human beings than has hitherto been appreciated; and that liberal intakes of vitamin C should be assured for surgical patients.

King, Musulin, and Swanson (1940) conclude that for the best interests of people generally, the need of maintaining a vitamin C intake "above that required for normal growth and external evidence of adequate nutrition is clearly indicated."

Gaetgens and Werner (1937) find evidence of decidedly increased vitamin C requirement during pregnancy.

The muscular exercise of a single prolonged athletic performance has been found to increase the body's rate of destruction of vitamin C and therefore its need of this factor.

Another fact bearing upon the problems of reasonable standards of ascorbic acid consumption is the increased production and consumption of fruits and fresh vegetables; for it now is no longer true that liberal consumption of fruits and their juices is a luxury and out of reach of the poor. Relatively low prices are practically en-

sured by the large orchard plantings now coming into bearing; and the newer knowledge of nutrition shows fruit to be a good investment up to consumption levels much higher than yet attained. Hence antiscorbutic food consumption levels considerably higher than have prevailed hitherto are economically feasible for the population as a whole, if we are convinced that research shows higher levels of vitamin C intake to be advantageous to nutritional well-being.

### Foods as Sources of Vitamin C

Table 47 shows the averages of available data for some typical foods, in terms of the milligrams of ascorbic acid contained in 100 grams of the edible part of each food in the condition (moist or dry) in which it is commonly purchased in the market or delivered to the consumer.

TABLE 47. — VITAMIN C IN CERTAIN FOODS: MILLIGRAMS PER 100 GRAMS

| FOOD                                   | NUMBER OF CASES | MEAN WITH ITS PROBABLE ERROR |
|--|-----------------|------------------------------|
| Lemon (or juice)                       | 37              | 56. ± 1.1                    |
| Orange (or juice)                      | 148             | 54. ± 0.55                   |
| Grapefruit (or juice)                  | 109             | 39.3 ± 0.44                  |
| Cabbage, raw                           | 18              | 35.0 ± 1.7                   |
| Strawberries                           | 16              | 34.4 ± 3.1                   |
| Cantaloupe                             | 70              | 29.2 ± 0.9                   |
| Turnip, fresh raw                      | 17              | 26.2 ± 1.8                   |
| Tomato (or fresh juice)                | 300             | 22.7 ± 0.41                  |
| Peas, fresh young, green, raw          | 120             | 22.4 ± 0.44                  |
| Asparagus, raw                         | 38              | 19.8 ± 1.4                   |
| Radishes                               | 13              | 15.7 ± 1.2                   |
| Beans, snap or string, raw             | 111             | 15.4 ± 0.39                  |
| Lettuce                                | 21              | 13.5 ± 2.17                  |
| Potatoes, raw                          | 82              | 12.6 ± 0.33                  |
| Corn, sweet, raw                       | 24              | 9.4 ± 0.24                   |
| Peaches                                | 26              | 8.7 ± 0.56                   |
| Bananas                                | 98              | 7.6 ± 0.20                   |
| Apples (medium varieties) <sup>a</sup> | 69              | 7.0 ± 0.22                   |
| Celery (stalks)                        | 22              | 6.8 ± 0.41                   |
| Watermelon                             | 100             | 6.6 ± 0.15                   |
| Plums                                  | 22              | 5.4 ± 0.46                   |
| Pears                                  | 18              | 4.1 ± 0.43                   |
| Carrots, raw                           | 16              | 4.0 ± 0.30                   |
| Milk                                   | 218             | 2.18 ± 0.018                 |

<sup>a</sup> Varieties here averaged: Astrachan, Baldwin, Ben Davis, Esopus, Golden Delicious, Gravenstein, King, Newton Wonder, Northern Spy, Rhode Island, Rome Beauty, Roxbury Russet, Spitzenburg, Stayman, Winesap, Winter Banana, and Yellow Newtown.

Among things which enter to an important extent into the American food supply, the citrus fruits and tomatoes are outstanding sources of vitamin C. They also hold their vitamin C values extraordinarily well. Canned grapefruit, canned grapefruit juice, and canned tomatoes have all been found, in repeated quantitative experiments, to show practically the same ascorbic acid contents (vitamin C value) as the respective fresh fruits.

Commercially canned tomato juice presents a different problem in that the factory method of pulping the tomatoes usually involves accidental admixture of air which may or may not be effectively removed before the canning.

Cantaloupes, strawberries, raw cabbage, and raw turnips rank about with tomatoes in ascorbic acid content; but cantaloupes and strawberries are usually much more costly than either tomatoes or citrus fruits, while cabbage and turnips must be expected to lose much more of their vitamin C value in cooking than do tomatoes. In England during the World War of 1914-18, when citrus fruits and tomatoes were scarce, raw turnip juice (particularly of the type of turnips called swedes) was much used as an antiscorbutic in feeding children.

Potatoes, fresh peas, and green leaf vegetables are also rich in ascorbic acid when raw, and while the cooked forms in which they are commonly eaten have suffered more or less diminution of vitamin C value they may still be important sources because of the quantities in which these foods may well enter into even low-cost diets.

Apples and bananas do not have as high an ascorbic acid content as do the aforementioned foods when raw, but as apples and bananas may be eaten raw in considerable quantities they are capable of playing important rôles as antiscorbutics. Under domestication the apple has been differentiated into many and widely different varieties, each bred for the accentuation of some particular characteristic, such as earliness, size, color, flavor, keeping quality, firmness under marketing conditions, etc. It now turns out that these widely differentiated varieties differ considerably in their vitamin C values. Data for about twenty medium varieties (*i.e.*, excluding both those exceptionally high and those exceptionally low) are included in the average here given.

In young snap beans, Mack, Tapley, and King (1939) found more of the ascorbic acid in the seeds than in the pods. No signifi-

cant differences in vitamin C value could be attributed to the type of soil upon which the snap beans were grown, or to the fertilization of the soil. The chief cause of difference in the freshly harvested crop was the age; the younger the snap beans, the higher their vitamin value. Also the more freshly they are used; for there is a marked loss in storage. Even at 1°-3° the present study showed 42 to 65 per cent of the vitamin C value lost in 6 days; at 8°-10°, 50 to 77 per cent; at 21°-23°, 58 to 81 per cent.

*Mature, resting seeds* evidently contain very little if any vitamin C. In a diet adequate but for vitamin C, the amount of grain fed has no appreciable effect upon the appearance or severity of scurvy. But if *properly sprouted grain or legume* be fed it is found to have pronounced antiscorbutic properties. As nothing but water (and air) need enter the seed, it is plain that something pre-existing in the seed must become transformed into vitamin C as the seed sprouts. This formation of vitamin C in the seed as it passes from the resting stage into a condition of active metabolism is undoubtedly of fundamental significance, and now that the chemical nature of vitamin C is known, elucidation will doubtless proceed rapidly.

In general, the studies of the distribution of this vitamin in plant materials show it to occur most abundantly in the actively functioning and the succulent parts, — in the fresh green leaves, the growing shoots, and the juicy stems, roots, tubers, bulbs, and fruits. It is to be mentioned, however, that in any such simple grouping there may be considerable variations within a group, and as yet (1940) this is not to be avoided by a more elaborate subdivision of plant products according to botanical classification or general chemical composition, since in some cases foods very similar from either of these points of view appear to have quite different antiscorbutic values, as illustrated by certain varieties of apples.

All of the commercial fats, sweets, and mill-products of grains are either entirely or practically devoid of vitamin C value.

Muscle tissue contains very little vitamin C. Repeated attempts to show the presence of vitamin C in ordinary meats by the usual tests have given negative or insignificant results. On the other hand, observations upon human scurvy have sometimes indicated that meat, if eaten sufficiently fresh, raw, or "rare," and in large quantities, has an appreciable though small antiscorbutic value. There is usually a somewhat higher concentration in liver. It would not appear safe, however, to depend upon cooked meat, as



ordinarily eaten, to furnish any significant amount of the antiscorbutic vitamin.

Eggs, like ordinary meats, seem to contain only traces of vitamin C.

According to Hess, a pint per day of average milk supplies the antiscorbutic requirement of a child. In view of our present belief that liberality of intake of vitamin C is highly beneficial, it is probably best to recommend that all infants be given some such richly antiscorbutic food as orange juice, and if this is done the possible variations in vitamin C content among the different forms of milk need not be a matter of practical concern. It is, however, well to know that the present-day, fresh market milk contains, whether raw or pasteurized, \* more vitamin C than is commonly supposed. Some early experiments with cows on rations of too artificial a character evidently gave incorrect impressions which have been widely disseminated even through some of the most authoritative writings.

Vitamin C is readily changed by oxidation and the greater part of the destruction which takes place upon heating under ordinary conditions is probably of an oxidative nature. Thus Kenny found that the rate of destruction upon heating which had previously been studied quantitatively by La Mer, Campbell, and Sherman can be reduced about two thirds by rendering the conditions of the heating experiment very rigidly anaerobic; and that the more rapid destruction of vitamin C in cabbage juice than in tomato juice, when both are at the same temperature and hydrogen-ion activity, may be correlated with the higher oxidation potential of the former medium.

Hence most of the destruction of vitamin C which occurs in such heating as may be applied to foods in household or industrial operations is in some sense due to oxidation; but it is usually as the result of heating that such destruction becomes measurable, and the careful quantitative studies which have been made upon the relation of temperature and hydrogen-ion activity to the rate of destruction of vitamin C (as illustrated in Fig. 30) are of permanent scientific and practical significance, whether we explain the chemical mechanism of the destructive process as wholly an oxidation or as somewhat more complex.

\* Holmes and coworkers (1939) found the loss in pasteurization to be less than 20 per cent.

With other conditions uniform, a difference in hydrogen-ion activity (pH; "reaction") may be expected to have a marked influence upon the stability of vitamin C.

In tomato juice at its natural acidity of pH 4.2 to 4.3, only 50 per cent of the vitamin C was destroyed on heating for 1 hour at 100° C.; but when the medium was brought to pH 9 before heating, the destruction was increased to 65 per cent. And even in a refrigerator at 10° C. the instability of the vitamin C at pH 9 was such that within 5 days the destruction just noted had increased to about 95 per cent.

To the frequent question whether "adding a little soda in cooking destroys the vitamin," the best answer would seem to be: Not necessarily all of it; but always more of the vitamin C is destroyed when soda is added than when it is not. The more soda added the more vitamin destroyed; this is true whether or not the change of pH caused is such as to shift it over the neutral point of pH = 7.

When the rate of destruction of vitamin C in tomato juice of

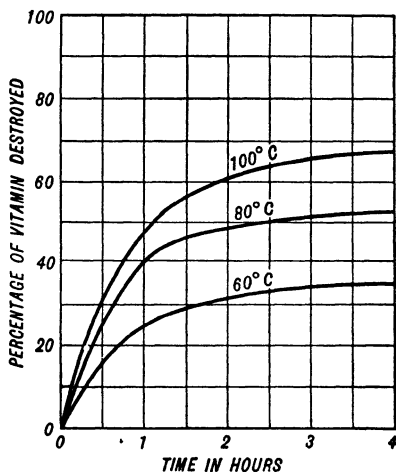


FIG. 30. — Curves representing the rates of destruction, at different temperatures, of the vitamin C of tomato juice at its natural acidity (pH = 4.3).

natural acidity (pH 4.2–4.3) was studied at the three temperatures 60°, 80°, and 100° C., it was found (as may be seen from Fig. 30) that both time and temperature are highly significant factors for consideration in any problem having to do with the stability of vitamin C and the extent to which it is likely to be destroyed in the practical handling of food materials.

We should also emphasize the fact that ascorbic acid is a freely soluble, readily diffusible substance so that in addition to what is destroyed in cooking or canning, there may be considerable further loss of vitamin C value if the cooking water or the liquid in the can is thrown away. See, for example, among the suggested readings which follow: Fenton and Tressler (1938); Fenton, Tressler, Camp, and King (1937); Fenton, Tressler, and King (1936).

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## CHAPTER XVIII

### THIAMIN (VITAMIN B<sub>1</sub>)

#### The Finding of the Nutritional Nature of Beriberi

THE DISEASE beriberi was long familiar to the Orient before it became well known to the Western world; so long and so widely, in fact, that the origin of the name is uncertain. Some trace it to one language according to which it means a great weakness; others to the word for sheep (in a different language) because the first outward sign is usually a stiffness of the ankles giving the victim a "sheep-like" gait.

In formal terminology it is (or begins as) a *multiple peripheral neuritis*, a disease of the nerves of both feet. In severe cases it may result in paralysis of the legs, and sometimes ultimately in heart failure.

In 1878-1883 the entire enlisted force of the Japanese navy numbered about 5000 men, and of these 1000 to 2000 each year were at some time on sick-list with beriberi. This enormous morbidity of 20 to 40 per cent from one disease led to an investigation by Takaki, a high medical officer of the Japanese navy, who worked upon the theory that the fault was most likely to be found in the food since climate appeared to be without influence and the sanitary conditions on the Japanese ships were as good as those in the European navies which were not troubled with the disease. A Japanese naval vessel with 276 men on a 9 months' cruise from Japan to New Zealand, Valparaiso, and Honolulu had 169 cases of beriberi, with 25 deaths. On another vessel with a similar crew, sent over the same route, but with a ration in which the rice was decreased, the barley increased, and vegetables, meat, and condensed milk added, only 14 men had beriberi and each of these had failed to eat his full allowance of the new foods. As a consequence of this experiment, Takaki secured the adoption of a new ration for the entire Japanese navy with the result that the number of cases of beriberi soon became practically negligible.

From his government, Takaki received full recognition for this

really great achievement; and yet it failed to accomplish for the world what might reasonably have been expected of it. Opinion continued to be divided, as it had been before, as to whether beriberi were a nutritional or an infectious disease, with the majority of the medical men of the Orient (where the disease continued to be common) inclining to the theory of an infection. Takaki, as we now know, was right in his belief that the disease was essentially nutritional and he was entirely explicit in saying so, but his work did not carry conviction because he offered no adequate theoretical explanation of it. He discussed his improvement of the ration only in terms of protein, which others rightly regarded as unconvincing. It was a time of great activity in the sanitary applications of the then new science of bacteriology. Sanitary improvements had naturally been made in the Japanese navy during the same years in which Takaki was succeeding in getting the ration changed, and the sanitary improvements were generally given credit for the eradication of the disease because the germ theory appeared adequate even with the germ unidentified, whereas Takaki offered no satisfactory scientific explanation of what he had accomplished.

In 1897, Eijkman, a Dutch physician working in the East Indies, published observations upon "an illness of fowls similar to beriberi." He had noticed that fowls living in the bare yard of the prison hospital, of which he was then the medical officer, and subsisting almost exclusively upon the left-over rice from the prisoners' table, often showed stiffness and weakness of the feet and legs very suggestive of that of his beriberi patients; and by systematic experimentation he found that this disease could regularly be induced in fowls by confining them strictly to a polished-rice diet. This is now generally regarded as the first clear-cut case of experimental production of a dietary deficiency disease. At the time, however, Eijkman's presentation and discussion of his findings was so strongly colored by what English investigators afterward called "the pharmacological bias" that the bearing of his work upon normal nutrition was not made clear. Reading Eijkman's early work in the light of later knowledge, one may take it as marking the initiation of our experimental knowledge of dietary deficiency diseases; but it was not until some years later, and until the experiments had been importantly extended by Grijns (1901), that the results of this work were stated in terms of a clearly nutritional concept.

Meanwhile British and American workers in the Orient were also beginning to give systematic attention to the nutritional aspects of beriberi.

For example, when American Army officers took over the administration of the Bilibid prison in Manila they sought to improve the treatment of the prisoners by furnishing them a better commercial grade of rice — plump, pure-white rice of highest market grade instead of the “low-grade” irregularly shaped brownish rice previously purchased. Shortly thereafter, the proportion of cases of beriberi in this prison population began to increase; but for some time this was regarded (it is to be remembered that the date was 1900–1901) as an epidemic to be combated by sanitary measures. After several months during which everything possible in the way of sanitation had been done, but without conquering the epidemic of beriberi, attention was turned to the “nutrition hypothesis”; dietary changes analogous to those which Takaki had made in the Japanese navy were tried; and the beriberi was then brought promptly under control.

At about the same time Fletcher, by experimenting with the diet in a hospital for the insane, showed that when 28 ounces of rice were fed daily with only small amounts of other food, the use of white polished or of brown unpolished rice was alone sufficient to determine the occurrence or non-occurrence of beriberi.

In 1909, convinced that beriberi was related to diet, the United States Army Medical Commission in the Philippines initiated changes in the rations of the Philippine Scouts and in 1911 Chamberlain was able to announce the eradication of the disease from these troops by the improvement of their ration.

In 1908–1909, when beriberi was at its worst among the Scouts, the ration consisted essentially of 12 ounces of beef, 8 ounces of white flour, 8 ounces of potatoes, and 20 ounces of rice (ordinarily polished). The change in the ration, as finally decided upon after some months of experimentation, consisted in giving, in place of the 20 ounces of polished rice, 16 ounces of unpolished rice and 1.6 ounces of dried beans. Experiments, made largely upon fowls, have shown that while beef has some effect in preventing beriberi, an equal weight of beans, peas, or peanuts is much more efficacious. Chamberlain states, in fact, that the disease had practically disappeared as the result of adding the beans to the ration, before the substitution of unpolished for polished rice had been completed.

He held, "that the consumption of beans to the daily amount of 1.6 ounces would, unaided, have prevented a recurrence of beriberi, but it would obviously be difficult to make sure that all the men ate their share of this article over long periods, and it is therefore much safer that the largest component of the diet, the rice, should be of the unpolished variety and by itself sufficient to prevent neuritis." Several other investigations gave similar results.

Repeated demonstrations of a close connection between diet and the occurrence or prevention of beriberi naturally gave a great impetus to experiments designed to find, and to identify chemically, the precise substance which has the property of preventing and curing the neuritis which is the outstanding feature of this disease.

### The Search for the Antineuritic Substance

As early as 1902, Hulshoff-Pol has shown that the antineuritic property could be demonstrated not only with the effective foods themselves, but also with extracts made from them, and even after clarification with basic lead acetate and subsequent removal of the lead.

Fraser and Stanton, working in the Malay States, and Chamberlain, Vedder, and Williams in the Philippines, together showed the antineuritic constituent of foods to be an organic substance more stable in acid than in alkaline solution, probably a nitrogen compound, but not identical with any known amino acid or alkaloid. Funk studied the substance further and named it *vitamin(e)*. Here the introduction of the name of the substance followed by about 10 years its clear apprehension as a definite chemical concept, and preceded by about 15 years its isolation by Jansen and Donath in 1926, while nearly another decade of research was required for Williams to work out a method of separating the pure substance in sufficient quantity to permit of the establishment of its molecular structure (full chemical nature).

### The Chemical Nature of the Antineuritic Vitamin

Space is not available for a description here of the many contributions to the isolation in crystalline form and the working-out of the chemical nature of this substance. It must suffice to say that larger-scale production was made possible by the method of

$$\begin{array}{c}
 \text{CH}_3 \\
 | \\
 \text{N}=\text{C}-\text{NH}_2 \cdot \text{HCl} \quad \text{C}=\text{C}-\text{CH}_2\text{CH}_2\text{OH} \\
 | \quad | \quad \quad \quad | \\
 \text{CH}_3-\text{C} \quad \text{C}-\text{CH}_2-\text{N} \\
 || \quad || \quad \quad || \\
 \text{N}-\text{CH} \quad \quad \quad \text{Cl} \quad \text{CH}-\text{S}
 \end{array}$$

After the molecular structure had been established, the name *thiamin* (thiamine chloride, thiamine chloride hydrochloride) was chosen as the official name of this substance by the Federal Food and Drug Administration, the American Medical Association, and the United States Pharmacopeia. It has also been adopted or endorsed by American scientific societies generally. The term vitamin B<sub>1</sub> continues to be frequently used synonymously with thiamin. The additional synonym, aneurin, is sometimes encountered in European literature; but its use in this country is definitely discouraged because of the "therapeutically suggestive" character of the word aneurin.

### Thiamin in Normal Nutrition

While earlier experiments of Lunin and of Pekelharing may now be seen to have dealt with what have since come to be called vitamins, it was the work of Hopkins which first made the concept effectively clear to most students of normal nutrition. His alcohol extracts of dried milk and of certain dried vegetables were clearly shown to contain some substance or substances essential to growth. We now know that these extracts contained both fat-soluble and water-soluble vitamins and that one of the latter was the substance now named thiamin.

Thus the longest-known functions of thiamin are the promotion of growth and the prevention of polyneuritis.

Thiamin also has an important function in relation to appetite.

While all or most nutritional deficiencies tend to result in gradual decline of appetite, thiamin deficiency causes (after a short period in which the body is presumably using-up its reserve) a characteristically more abrupt loss of appetite than does any other known vitamin deficiency. Also, thiamin promotes the recovery of appetite in a more prompt and specific way than does any other known nutrient.

And it is not simply that this vitamin makes the food more appetizing: it is an effect of thiamin upon the appetite as a physiological function. For even the feeding of the thiamin *separately* causes the animal to return with appetite to the food which it had refused.

Such observations gave currency to the unqualified statement that this vitamin stimulates appetite. More recently there has been a tendency from two points of view to qualify this statement. From the viewpoint of what is "permissible to claim" it has been proposed to limit the claim for thiamin's action on appetite to a statement that it promotes recovery of an appetite which has been lost because of its lack. And in answer to the question whether stimulation of appetite is normally desirable it has been said that thiamin promotes recovery from a condition of subnormal appetite; and stabilizes the appetite at the normal point, or degree of intensity.

It may perhaps be questioned whether the evidence fully supports these somewhat drastic limitations upon the hitherto accepted simple statement that one of the functions of this vitamin

is to promote the appetite. Certainly some scientifically-trained people have thought they observed increases in already-normal appetites after taking thiamin. And it seems reasonable to suppose that a positive promotion of appetite is involved in the greater growth which results from the higher levels of thiamin feeding among animals normal throughout, as described below and illustrated in Fig. 31.

Thiamin seems also to function in the maintenance of the normal motility of the digestive tract. Both L. J. Harris and M. S. Rose speak of a general lack of tone of the gastro-intestinal tract as resulting from a shortage of this vitamin. Cowgill, experimenting with dogs, observed a diminution of vigor in the normal contractions of the stomach as an earlier result of thiamin shortage than the loss of appetite. As the loss of normal motility may extend to the intestine, the frequently reported constipating effect of diets too largely composed of artificially refined food may perhaps be partly due to paucity of thiamin.

*Thiamin functions very importantly in the intermediary metabolism.* An excellent series of experiments by Peters and his coworkers at Oxford has shown that thiamin, presumably acting mainly in the form of its pyrophosphate (cocarboxylase), catalyzes the transformation of pyruvic acid and thus helps the carbohydrate metabolism through its intermediary stages. Thus thiamin carries the intermediary metabolism of carbohydrate through an essential step, whether on the way to combustion as fuel or to conversion into fat. This shows thiamin to be a substance of great importance to the working of the active cells in all the bodily systems.

Williams and Spies (1938) emphasize this repeatedly and hold that it affords a scientifically satisfactory explanation of the clinical reports of helpful results from thiamin therapy in a very wide variety of ills.

They also suggest that the abnormal accumulation of pyruvic acid (and perhaps also other intermediates) in the body when thiamin intake is too low may be the cause of failure of appetite and of decline in gastro-intestinal motility and general bodily tone.

To what extent the apparent "toning-up" property of thiamin is limited to "curing the consequences of a previous lack of the same substance," and to what extent it stimulates an already normal appetite and bodily tone, is not yet entirely clear.

In laboratory experiments in which other conditions were con-

stant while young animals were fed different amounts of thiamin-containing food, a basal diet adequate in other respects being always available to them, appetite and growth continued to rise with thiamin intake to levels well above the absolute (or minimal) needs of health (Fig. 31). Since the thiamin was here given in a natural food form, it is possible that other factors may have played some part in producing the result shown.

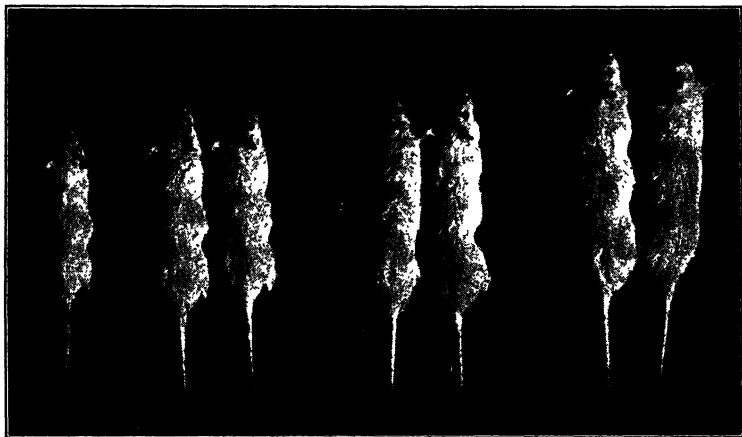


FIG. 31. — Effects upon growth of feeding four different levels of thiamin as explained in the text. (From experiments by Dr. Bertha Bisbey.)

There is abundant evidence that “the vitamin-B complex” is importantly concerned in the nutritional support of successful pregnancy and lactation; but just how this responsibility is shared between thiamin and riboflavin (and conceivably still other factors) is still a problem for further research.

#### Quantitative Determination of Thiamin

Methods in wide variety have been proposed for the determination of thiamin.

Some of them are mentioned here in an order which follows approximately the chronological sequence in which they were developed. The first two are based on feeding experiments of the same general type as those through which the existence of thiamin was discovered and its concentration and isolation from natural sources was guided. At the other end of the list are the *in vitro* methods whose development could not even be begun systemati-



cally until pure thiamin was available in sufficient abundance to be used freely as reference material in analytical work.

*Methods Utilizing the Influence of Thiamin upon Growth  
of Mammalian Species*

Rats served as subjects in the work of Hopkins, of Osborne and Mendel, and of McCollum which established the existence of this among new factors as essential to normal nutrition, and the growth methods for its determination have continued to make use of this species.

Such quantitative feeding tests can also readily be made to furnish striking demonstrations of both the prevention and cure of nutritional polyneuritis by thiamin and visible evidence of its effect upon growth.

Thus in experiments made to measure the vitamin B value of food, rats of the same litter and essentially the same initial size may be fed (in addition to a basal ration good in all other respects) graded amounts of thiamin in the form of the food under investigation. In a typical case two rats each received three different levels (daily allowances) while the seventh member of the litter received the smallest daily amount. All received enough to be protected from polyneuritis, but their rate of growth was restricted in regularly graded degrees according to the amount of the thiamin-containing food allowed in each case (Fig. 31). Noticeable also is the good agreement between each two test animals receiving the same amount.

While the effect on the weight curve is usually the best basis for *measurement*, the most *characteristic effects* of shortage of thiamin are failure of appetite and development of polyneuritis. As the appetite fails there is also a development of much the same condition as is produced by starvation. *Complete* deprivation may even result in death without the appearance of the characteristic nerve symptoms.

Thus in the accompanying set of weight curves taken from the work of Dr. Chase (Chase and Sherman, 1931) the rats which were given no thiamin whatever are seen to have died before there was time for them to develop polyneuritis. Or perhaps it would be more strictly accurate to say that before there was time for typical polyneuritis to develop the animals had become too weak and moribund for a satisfactory diagnosis of polyneuritis to be made;

at any rate the typical or characteristic nerve symptoms did not become fully apparent. When, however, life was prolonged by the feeding of a *very* small, decidedly inadequate amount of thiamin, the typical nerve symptoms appeared, the time required for the appearance of polyneuritis tending to vary with the daily intake of thiamin, even among intakes all of which were decidedly inadequate (Fig. 32).

At a level of feeding of the test food which furnishes enough thiamin to permit the standardized rat (previously depleted of any body surplus of this vitamin) to grow at a rate of about 3 grams per week, the animal is also protected from the polyneuritis. This level of limited growth and protection from the characteristic deficiency disease has frequently been made the basis for quantitative comparisons of thiamin values of foods. Comparisons on the same principle can, of course, be made at higher levels (rates of growth) if preferred. *In all quantitative work, "positive control" animals receiving known amounts of thiamin, as pure substance or as International reference material, are to be fed and kept side-by-side with the animals receiving the graded amounts of the food or foods under investigation.*

Many details may now be left to individual preference provided the work is done by sufficiently trained and experienced people and includes sufficiently close and numerous parallel feedings with pure thiamin. Booher and Hartzler (1939), after consideration of

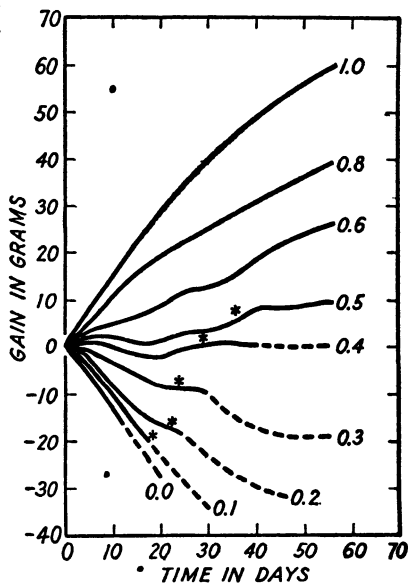


FIG. 32. — Average growth curves of rats receiving different amounts of wheat as sole source of thiamin. The amount of wheat fed six times weekly is shown in grams at the end of each curve. Twelve rats were fed at each level. Broken lines indicate the continuing record of the survivors after one or more had died.

\* Indicates the point at which, in the average of the animals fed at the stated level, distinct polyneuritis appeared. (Courtesy of the *Journal of the American Chemical Society*.)

other methods, adopted this rat-growth method for their determinations of thiamin contents of foods.

*Methods Based upon the Prevention or Cure of Polyneuritis in Birds*

The fact that the experimental demonstration of the nutritional nature of beriberi was first accomplished with birds, gives such experiments an historic place coordinate with that of the rat-growth method. Critically regarded from the quantitative viewpoint, however, birds have in most laboratories appeared somewhat less uniform in their responses to the feeding of graded amounts of food than do the rats of a well-controlled colony. Peters has developed at Oxford a highly special technique of feeding and selection which provides for the use of only such pigeons as show a pure head-retraction type of experimental polyneuritis, the cure of which under controlled conditions is used as a means of measuring the potency of antineuritic vitamin preparations.

*Methods Based on the Cure of Thiamin-Deficiency in Rats*

These methods are of two types: (1) *the bradycardia method*, based on the slowing of the heart rate in rats by thiamin deficiency and its cure by test doses of food under controlled conditions; and (2) *the U. S. Pharmacopeia method*, based on the previous work of McCollum, of Smith, and of Church, in which a standard grade or degree of polyneuritis is produced in the rat and cured by test doses of the material under investigation.

*Methods Utilizing the Effect of Thiamin on Microorganisms*

The marked influence of thiamin upon the growth, multiplication, or fermentative activity of a suitable species of microorganism obviously offers an attractive basis for economical determinations of thiamin values. Several such methods have been suggested and at least one is being used for control work in certain food industries. The validity of a method of this type will depend not only upon its specificity but also upon the accuracy with which all the other conditions which influence the behavior of the microorganism can be controlled. Such a method may, therefore, have been sufficiently developed to yield useful results in comparative tests upon foods of one type, without being equally dependable for application to foods of all types.

*Strictly in vitro Methods for the Determination of Thiamin*

The suggestion just given regarding methods using microorganisms are also applicable, at the moment of writing (1940), to the strictly *in vitro* methods of determining thiamin. Work upon the further development and testing of such methods is, however, both active and promising, so that it is readily conceivable that, by the time this is read, one or more *in vitro* methods may have reached an acceptable stage of development and tested dependability, for use in measuring the vitamin values of foods generally.

**Quantitative Expression of Thiamin Values**

Whatever the method employed in measuring thiamin values, good usage now requires that all quantitative testing include experiments with known amounts of the vitamin side-by-side with those upon the food or other material under investigation.

When properly conducted and interpreted such quantitative experiments permit statement of the thiamin value found in terms of the weight of the actual substance (thiamin, thiamine chloride, or thiamine chloride hydrochloride).

Williams and Spies (1938) recommend strongly that henceforth all statements of thiamin values be made in terms of the weight of the substance and not in terms of any conventional "unit."

This plan is being followed increasingly and will be used in this book. *The microgram* (millionth of a gram, often represented by the Greek letter gamma or by  $\mu\text{g}$ ) per 100 grams of food is the most convenient scale of expression.

By resolution of the vitamin committee of the Health Organization of the League of Nations, the "International Unit of vitamin B<sub>1</sub>" is now three micrograms of the actual substance.

**Human Requirements for Thiamin**

Williams and Spies' (1938) extended and critical studies, both in the correlation of food records with incidence of beriberi and in the experimental evidence as to the mode of functioning of thiamin in nutrition, lead to the conclusion that the thiamin requirement is proportional to the *non-fat* Calories of the food or of the metabolism.

Undoubtedly this is more strictly in accordance with the scientific evidence than to try to fix thiamin requirement in terms of total Calories as had previously been more or less customary.

If, for convenience, one still sometimes speaks of thiamin requirement as so many micrograms per 100 Calories of food, it should be recognized that this involves a relatively large "simplifying assumption" which can no longer be regarded as literally true.

A much better simplifying assumption is accepted when one bases the calculation of thiamin need upon non-fat Calories. The actual scientific explanation is, of course, that the thiamin functions in carrying the intermediary metabolism through the pyruvic acid stage. *But a fraction of the energy value of fat resides in the glycerol radicle which does go through this same step in its intermediary metabolism and a fraction of the energy value of protein is in certain amino acid radicles which presumably do not pass through the form of pyruvic acid when metabolized.* An assumption that these two fractions approximately balance each other is involved when one adopts "non-fat" energy as a quantitative basis for estimating thiamin need.

Williams and Spies (1938) reached the conclusion that one needs between 27 and 30 micrograms of thiamin per 100 non-fat Calories.

If then a normal adult's daily food furnishes 800 to 1000 Calories as fat, and 1600 to 2000 Calories from other sources, the corresponding thiamin need would be about 400 to 600 micrograms per adult per day.

It will be noted that this estimate already contains an allowance for safety. For, whereas our estimates of protein, phosphorus, and calcium requirements start from a figure which represents average need, the thiamin estimate starts from a figure representing the requirement of those who needed most. This is because if *any* of a given group or population receiving a certain food supply showed evidence of needing more thiamin, the observation was counted as showing that a higher thiamin level was needed. As a matter of fact, this constitutes a larger allowance for safety than does the 50 per cent which has so often been added to the average laboratory finding of minimal need in estimating "requirement" of protein, or of phosphorus, or of calcium.

Hence an allowance of 400 to 600 micrograms of thiamin per day for a normal adult, depending upon size and activity, is as generous as the allowances in general use as "standards" for protein, phosphorus, or calcium.

Inasmuch as children, like adults, customarily take (in this coun-

try, at least) about one third of the food calories in the form of fat, the thiamin allowance for a child or a family may be taken as bearing the same relation to total Calories as 600 micrograms of thiamin with 3000 total Calories but current (1940-41) views tend to higher allowances as desirable.

In pregnancy and lactation women are deemed to need from one-fourth to one-half more than they need at other times.

### **The Problem of Optimal Intake of Thiamin**

A beginning has been made in the study of the urinary output of thiamin as index of the degree of "saturation" of the body with this substance, on the general principle that has been so much studied in the case of ascorbic acid. As yet, however, there is not adequate scientific evidence that the body would be benefited, in the long run, by being kept saturated with thiamin. One pediatrician has suggested that to feed a baby on such an assumption would be like continual feeding with over-salted food; but most recent discussions tend to assume that quite high levels of thiamin intake are beneficial. Poole, Hamil, Cooley, and Macy (1937), for instance, hold that liberal thiamin intake stabilizes the appetite at about its optimal point.

The question whether the best level of intake is that which keeps the body-concentration near the top, near the middle, or near the bottom of the normal zone, is a problem for experimental study in the case of each nutritional factor separately.

There is great need of a thorough investigation of the influence of different liberal levels of thiamin intake, continued throughout the life-times of at least two generations (preferably more) of laboratory animals.

The general acceptance of the theory that thiamin in some sense stimulates the active cells of all the body systems, and the apparent disposition to assume that such stimulation is beneficial, make it important that we should know what degree of liberality of thiamin consumption is conducive to highest health throughout a life-time and successive generations.

While no other evidence can satisfactorily take the place of full-life, successive generation experiments with animals under accurate laboratory control, it is possible that with thiamin as with vitamin C quantitative measurements of urinary excretion, especially if paralleled by determination of the concentrations of the vitamin in

blood and perhaps other bodily fluids and tissues, may throw light upon the short-range problem of "body saturation." Papers relating to such work will be found in the reading list at the end of the chapter; and doubtless others will have appeared by the time this book reaches the reader. In this connection it is well to keep in mind that methods for the measurement of thiamin in such materials are still in course of development, and that, as Najjar and Holt (1940) point out, both renal function and the interrelationships of thiamin with other vitamins may influence the amounts found in the urine.

Aykroyd and coworkers (1940) recommend a thiamin intake of 1.5 to 2 milligrams per day.

### Foods as Sources of Thiamin

Thiamin is widely distributed among natural foods of all types, and it is only the undue dependence upon artificially refined foods that makes the adequacy of the thiamin intake a practical problem in normal nutrition.

The same refining processes which impoverish foods in their thiamin contents lower also their values as sources of other vitamins of "the B group" and of mineral elements which are important in nutrition. The newer knowledge of nutrition should be so diffused as to make clear to all that even widely varied artificial flavoring of a dietary of over-refined foods can never be as good a practice as the use of a larger proportion of our foods in reasonably natural forms. The arts of cookery should be concerned more largely with natural foods; and not made a means of deceiving the palate with sophisticated foods which do not bring the body the needed mineral elements and vitamin values. Cookery can now be guided by nutritional knowledge to a much fuller degree than hitherto.

To what extent the thiamin content is diminished in cookery will doubtless depend both upon the kind of food in question and the exact nature of the cookery process chosen. The latter varies so greatly that the only scientifically satisfactory approach in the study of food values is to consider first the composition of the food as obtained from the land or from the market, leaving cookery losses for consideration (if deemed necessary) as a separate and subsequent step.

Table 48 herewith, and the more extended table in the Appendix,

show, therefore, estimates of average thiamin values of foods as obtained in the market or as delivered at the door, based on all the evidence available up to about the middle of 1940.

TABLE 48. — THIAMIN IN TYPICAL FOODS: MICROGRAMS PER 100 GRAMS

| Food   | RANGE WITHIN WHICH AVERAGE<br>WILL PROBABLY BE FOUND |
|--|--|
| <i>Foods of Animal Origin</i>                  |  |
| Beef muscle . . . . .                          | 110-210  |
| Chicken (and fowl) . . . . .                   | 90-380   |
| Lamb (and mutton) . . . . .                    | 200-300  |
| Pork muscle . . . . .                          | 700-1400   |
| Kidney (of cattle, sheep, and swine) . . . . . | 400-500  |
| Liver (of cattle, sheep, and swine) . . . . .  | 300-420  |
| Milk . . . . .                                 | 40-65  |
| Eggs . . . . .                                 | 140-160  |
| Egg white . . . . .                            | trace  |
| Egg yolk . . . . .                             | 350-440  |
| <i>Grain Products</i>                          |  |
| Oats (oatmeal) . . . . .                       | 345-770  |
| Rice, entire . . . . .                         | 240-300  |
| Rice, white (polished) . . . . .               | 30-40  |
| Wheat, entire . . . . .                        | 500-660  |
| White flour . . . . .                          | 60-100   |
| White bread . . . . .                          | 55-85  |
| Whole wheat bread . . . . .                    | 240-400  |
| <i>Dry Legumes</i>                             |  |
| Beans, pea or navy, dry . . . . .              | 315-510  |
| Beans, Lima, dry . . . . .                     | 450-600  |
| Peas, dry . . . . .                            | 300-620  |
| Peanuts . . . . .                              | 500-600  |
| <i>Other Vegetables and Fruits</i>             |  |
| Apples . . . . .                               | 20-55  |
| Bananas . . . . .                              | 50-100   |
| Beans, snap or string . . . . .                | 55-95  |
| Cabbage . . . . .                              | 70-140   |
| Carrots . . . . .                              | 60-140   |
| Lettuce . . . . .                              | 50-125   |
| Orange (or fresh juice) . . . . .              | 75-145   |
| Peas, fresh young green . . . . .              | 270-495  |
| Potatoes . . . . .                             | 95-165   |
| Spinach . . . . .                              | 95-155   |
| Tomato (or fresh juice) . . . . .              | 70-115   |



Perhaps the fact deserving of most emphasis here, because of intrinsic importance and because it is not usually recognized, is the wide distribution of thiamin in fruits and vegetables generally in concentrations which look small at first glance (largely because of the high water content of most fruits and vegetables) but which become of much importance to the daily nutritional intake when these foods are given as prominent a place in the dietary as they should occupy in the light of the newer knowledge of nutrition.

One has almost unlimited choice among fruits and fresh vegetables which with their natural high water content contain something of the order of 100 micrograms of thiamin per 100 grams of food. The casual reader, or even the author of an influential book, may think "only about one part in a million" and dismiss it as insignificant, but it should not be insignificant by any means.

A normal adult may well eat a total of at least a kilogram (35 ounces) of fruits and vegetables a day. If their average thiamin content is one part per million, this will mean that such an adult gets a *milligram* of thiamin a day from fruits and vegetables unless they lose unexpectedly large amounts in cooking.

Let us look then at the thiamin contents of typical individual fruits and vegetables.

According to the evidence thus far available, apples, bananas, cantaloupes, turnips, and watermelons contain less than one part per million of thiamin, but beans, cabbage, kale, oranges, peas, potatoes, and turnip greens contain more; so the round figure of about a milligram of thiamin per kilogram of fruits and vegetables consumed is evidently of the correct general order of magnitude. As for losses in cooking, apples, bananas, cabbage, celery, lettuce, peaches, pears, and tomatoes are largely (and well might be even more largely) eaten raw, while citrus fruits and melons are almost entirely eaten raw. Even tender young peas are almost always cooked (why?) but their initial thiamin content is so high that they may be expected to contain well over one part per million even after drastic cooking.

Dry legumes and whole-grain cereals and breadstuffs are of high thiamin value. White flour, white rice, and "new process" corn meal contain but little; and commercial sweets and fats, practically none.

Both meat and milk are shown by recent work to contain more

thiamin than has generally been supposed. Eggs also are a good source.

Here as elsewhere in this book, general statements about meats refer to the muscle meats because in the nature of things these must so greatly predominate in quantity over the glandular organs. The much higher concentration of thiamin in pork muscle than in beef muscle seems now to be definitely established.

### **Do Our "Customary American Diets" Contain Enough Thiamin?**

Abundance of thiamin in the dietary of an individual, or the food supply of a family, an institution, or a nation is very easily obtained whenever and wherever food habits are guided by nutritional knowledge.

This may be stated with confidence because of the now well established wide distribution of thiamin in significant amounts in both plant and animal organs and tissues. Food crops as the farmer sends them to the market or to the miller contain plenty of thiamin.

In so far as thiamin is a practical problem in the feeding of normal people, such a situation is due to wastes which are easily avoidable, too great dependence upon artificially refined foods, or to a combination of these factors.

If (1) at least half of the needed calories are taken in the form of such "protective" foods as milk (including cream and ice cream), fruits, vegetables, and eggs, and (2) at least half of the breadstuffs and cereals consumed are taken in the form of "whole-grain," "dark," "unskimmed" products, excellent provision will have been made for thiamin, and for other important vitamins and mineral elements at the same time.

It may be noted that this recommendation leaves wide scope for individual preferences even in that half of the dietary with which it deals; and, that up to one half of the total energy requirement is left to be satisfied by entirely unlimited choice among whatever foods the individual consumer finds most acceptable to his palate, his purse, and his digestive powers.

So the answer to the question whether "customary" diets provide ample intakes of thiamin will depend upon the prevalence and rate of spread among the people generally of such food customs as represent the reasonably intelligent use of the newer knowledge of nutrition. Note too, that people who have not the background

of scientific training to understand the experimental evidence which supports this nutritional knowledge may still use the teachings of the science of nutrition in deciding upon what for them is due prominence of fruits, vegetables, and milk in their individual or family dietaries.

If one is not prepared to think at all in terms of the "distribution of the calories" among the different articles or groups of foods, one may think in terms of the food budget and rest assured that fruits, vegetables, milk, cream, and ice cream are better investments and should constitute a larger fraction of the food budget than hitherto supposed.

Also, anyone, regardless of the extent of scientific training and regardless of whether one eats much bread or little, can make a custom of having at least half of the bread brown or approximately "whole grain."

Hence the answer to the question with which this section is headed is doubtless changing, and becoming increasingly affirmative.

There was a period of at least a generation after highly milled products had obtained an undue dominance in the breadstuffs supply, while per capita consumption of refined sugar was still increasing, and before the nutritive importance of a liberal consumption of fruits and fresh vegetables was known, when the customary American diet was probably too low in thiamin to be now regarded as satisfactory. Doubtless too, some people are still living according to the customs of that period. But undoubtedly it is also true that already our food customs are changing for the better. And, as above explained, the change for the better can easily and agreeably be carried further.

Notwithstanding the fact that wholewheat flour is a more perishable product than white flour, and that wholewheat bread has been too generally regarded as a "specialty," still it is sufficiently a staple so that it costs only about one tenth per pound more than white bread. And if the ingredients other than flour are the same for the two breads, the wholewheat bread for 10 per cent more cost, gives probably at least 300 per cent more thiamin and from 100 to 300 per cent more of several other vitamins and mineral elements. This statement is purposely put in a rather elastic form because enrichments of bread by use of more milk solids and by the use of high-thiamin yeast just now (1940) being introduced may influence

the quantitative ratios as between the two types of bread; but *with other ingredients alike*, the bread made of wholewheat flour will necessarily be superior on some points of nutritive value, to the bread made of white flour.

### "Fortification" of White Flour

At the time of writing (summer of 1940) the fortification of white flour by addition of synthetic thiamin and probably also some other enrichments is being actively debated as a problem of public policy. See Suggested Readings and look for later publications.

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## CHAPTER XIX

### RIBOFLAVIN

(formerly called *Lactoflavin*, *Flavin Factor*, or *Vitamin G*)

#### Molecular Constitution and Forms of Occurrence

*Riboflavin* is the name, formally adopted by the American Medical Association, the Society of Biological Chemists, the American Institute of Nutrition, and the Federal Food and Drug Administration, and rapidly coming into general use, for the water-soluble yellow substance with greenish fluorescence, which as isolated from milk was called lactoflavin.

*Lactoflavin*, *ovoflavin*, and *hepatoflavin* (all recognized as belonging to the group of organic substances known as flavins, and each named according to the natural source from which it was obtained) turned out to be identical, and the same substance became readily available as a synthetic product.

Its molecular structure and corresponding full name are shown in Fig. 33.

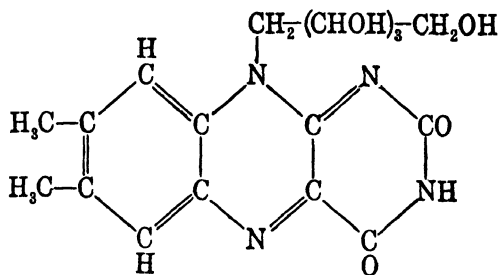


FIG. 33. — Riboflavin: 6, 7, dimethyl-9-d, 1-ribityl-isoalloxazine.

Riboflavin (sometimes free, sometimes in combination with phosphoric acid, often in combination with both phosphoric acid and protein) is very widely distributed in both plant and animal tissues. The so-called respiratory enzyme (Warburg's yellow enzyme) is a combination of riboflavin phosphate with protein. Several such

combinations are now known. The working-out of their interrelationships as catalysts of the oxidation processes involved in the energy metabolism of the tissues is an active field of research.

The fact that it has been identified in such diverse products of life as milk, eggs, liver, muscles, eyes, leaves, flowers, fruits, and seeds, and in both fresh-water and salt-water algae, raises the presumption that it is almost if not quite universally involved in the life processes of active cells.

The riboflavin-phosphoric acid-protein combination of typical yellow respiratory tissue enzyme is described by Kekwick and Pedersen (1936), on the basis of sedimentation, diffusion, and electrophoresis measurements, as a chemical entity with a molecular weight of the order of about 80,000 and with one flavin group in such a molecule.

In milk, the riboflavin exists partly as an analogous compound, but also very largely in readily diffusible form, evidently free from combination with any large protein molecule.

Whether riboflavin in food is in the free state, or in combination with phosphoric acid, or with phosphoric acid and protein, probably makes little difference to its nutritive value. According to one view, riboflavin, if free when it reaches the intestine, is combined with phosphoric acid in the course of its absorption through the intestinal wall.

Hence statements of riboflavin values of foods do not distinguish as to whether, or in what proportions, it exists in a free or a combined form. It is, however, to be kept in mind that some of the methods by which it has been sought to measure riboflavin *in vitro* have failed to include that present in combination (or in some of its combinations), so that uncritical compilations of data for riboflavin may include some values which are much below the true amounts.

### Significance for Nutritional Wellbeing

*The Journal of the American Medical Association* has spoken of riboflavin as "essential to the defense powers of the organism"; and Pinkerton and Bessey (1939) working in the Harvard Department of Pathology have shown that the level of nutritional intake of riboflavin greatly affects the susceptibility of experimental animals to rickettsial diseases.

There is abundant evidence that the amount of riboflavin in the

food has a large influence upon morbidity and mortality rates in laboratory animal populations, and strong reason to suppose that the same is true of human populations under the conditions that exist in this country and in most other countries.

Day and his coworkers showed that riboflavin deficiency causes deterioration not only of the skin but also of the eye (which by origin is a specially developed skin-spot). The correlation of the skin and eye conditions with each other and with cessation of growth in the young, and the experimental conditions under which these three signs of deficiency are governed by riboflavin, were clearly developed in the independent series of papers by Day and by Bessey with their respective coworkers. Their discovery of the sensitiveness of the eye to shortage of riboflavin, made by means of controlled experiments with laboratory animals, has since been strikingly confirmed in the clinical experience of Sydenstricker, Sebrell, Kruse, Cleckley, and others. Several references to this work upon riboflavin deficiency as a human disease will be found among those listed at the end of the chapter, and the reader will doubtless also find additional papers in later literature; for riboflavin deficiency as a human disease, often unrecognized because masked by pellagra, is being actively investigated at the time this is written.

Direct clinical evidence that riboflavin is nutritionally essential to man, and that riboflavin-deficiency is not only a possibility but an actually occurring disease in the United States, is furnished by the work of Sebrell and Butler (1938), Sydenstricker *et al.* (1939), Oden, Oden, and Sebrell (1939), Kruse, Sydenstricker, and Sebrell (1940), Sydenstricker, Sebrell, Cleckley, and Kruse (1940) and others listed among the references and suggested readings at the end of the chapter. \* *Ariboflavinosis*, as the riboflavin-deficiency disease has been named, occurs both independently and in conjunction with pellagra. In the latter cases, the much more dramatic symptoms of the pellagra, together with the fact that the two diseases usually both yield promptly to good diet, make it likely that a large proportion of the riboflavin deficiencies are overlooked. For much the same reasons it is probable that the pathology of ariboflavinosis as thus far known (1940) constitutes the beginnings

\* Here, as elsewhere, the reader may well keep in mind the possibility that a library search might reveal important publications which have appeared subsequently to the formulation (in 1940-41) of the reading-lists contained in this book.

rather than a complete picture of the consequences of riboflavin deficiency.

Cracking of the lips was the symptom emphasized by Sebrell and Butler in the first description of riboflavin deficiency as a human disease.

The ocular manifestations reported by Bessey and Wolbach (1939), and by Kruse, Sydenstricker, Sebrell, and Cleckley in January 1940, and described more fully by Sydenstricker, Sebrell, Cleckley, and Kruse in June 1940, are of outstanding interest among the specific symptoms of riboflavin deficiency so far as known at the time of the present writing (August 1940). The latest of the papers just mentioned (Sydenstricker, Sebrell, Cleckley, and Kruse, 1940) should be read by all who are interested in the symptoms. Slit-lamp examination showed an abnormal vascularity technically designated as "a superficial vascularizing keratitis" with "congestion and proliferation of the limbic plexus" as the earliest and universal symptom in a series of 47 cases, all of which were shown to be riboflavin deficiencies by the readiness with which they were cured by riboflavin alone. Some of these people were hospital patients who had been on "pellagra-producing" diets but were saved from the pellagra symptoms as now understood by means of nicotinic acid as will be explained in the next chapter. Others of the people in this series of 47 were members of the hospital staff who considered themselves well nourished and who came under observation merely because of cracked lips, eyes unduly sensitive to light, dimness of vision, or "eyestrain." While the 18 hospital employees chose their food at will from an apparently adequate food supply, investigation revealed that: "Bad dietary habits with inadequate intake of milk, eggs, and green vegetables were prevalent in the entire group." This investigation adds very strong evidence for the view that a large proportion of people need a different emphasis in their choice of food in order to get enough riboflavin to support them at the level of health of which they are capable.

Thus whether or not ariboflavinosis is an important disease, it is clear that riboflavin is an important factor in health, and in the building of already-normal health to higher levels.

Riboflavin is essential to growth, and to normal nutrition at all ages. When the food is poor in riboflavin for any considerable length of time, digestive disturbances, nervous depression (dif-

ferent from the symmetrical polyneuritis of thiamin deficiency though both may occur in the same person), general weakness and deterioration of tone, and poor conditions of the eyes and skin are apt to develop; the incidence of infectious disease is likely to be increased, vitality diminished, life shortened, and the prime of life seriously curtailed by the unduly early development of the physical manifestations of old age.

The converse picture is equally true and more attractive.

As compared with the results of a merely adequate or minimal-adequate intake of riboflavin, a diet of higher riboflavin value seems to result in better development, higher adult vitality, greater freedom from disease at all ages, somewhat longer life, and (more significantly) a longer "prime of life," *i.e.*, an extension of that segment of the life cycle between the attainment of adult capacity and the onset of old age.

This extension of the "period of the prime," — a property not of riboflavin alone but of the superior nutritional condition or internal environment in which riboflavin is apparently one of the major factors, — has been especially emphasized by Simms as important both to the individual and the community because it means longer life with *a smaller percentage of years of dependence*.

In controlled experiments extending through two generations of laboratory animals \* it has appeared: (1) that riboflavin ranked with calcium and vitamin A as the major factors in the improvement of nutritional wellbeing which resulted from an increase of the proportion of milk in an already-adequate diet; and (2) that in the experiments thus far completed in which riboflavin was the sole significant variable, successive increments of intake resulted in successively increased benefits to nutritional wellbeing, up to levels more than twice that of minimal adequacy. Whether the plateau of optimal benefit is reached at three or four times the minimal-adequate intake or whether there is further gain, measurable in the vitality of the offspring and reflecting a further superiority of internal environment of the adults, up to levels of intake perhaps seven to ten times that of minimal adequacy is still a subject of active research. Fuller discussion may be found, if desired, in several of the papers listed at the end of the chapter, *e.g.*, Sher-

\* It is probably unnecessary to explain that in this research, as in analogous cases cited elsewhere in this book, the species of experimental animal is chosen for the close resemblance of its nutritional chemistry to that of man, with reference to the particular nutritional factor under investigation.

man and Campbell (1924, 1930, 1937) and Sherman and Ellis (1934, 1939). We shall recur in a later chapter to the further consideration of the influence of differences of nutritional intake, above the level of mere adequacy, upon the internal environment of the normal body.

### **Problems of Interrelation of Riboflavin with Other Factors in Tissue-Respiration Metabolism**

At the time of writing (1940) the occurrence and significance of riboflavin in its different combinations and its possible interrelations with analogous derivatives of thiamin and of nicotinic acid \* are still subjects of such active research that an attempt at comprehensive summary here might be outmoded by the time it reached its readers. Axelrod, Sober, and Elvehjem's 1940 paper opens with a concise review of the situation in April of that year.

For the purposes of this book, it seems sufficient to call attention to the fact that the findings of several investigators† show that compounds (we do not yet know how many) of thiamin, of riboflavin, and of nicotinic acid all function in the complex enzyme-coenzyme systems which catalyze the oxidation process in the body tissues, and that there may be interrelations in their action. It is partly through studies of the enzymic reactions *in vitro* and partly through very comprehensive feeding experiments under exceptionally accurate laboratory control that one may hope to obtain further light upon such questions as: to what extent do the thiamin, the riboflavin, and the nicotinic acid compounds function independently; to what extent are any of their functions interlinked, so that shortage of one impairs the functioning of another; or do any two of them act in parallel to a sufficient extent that an abundance of one may in some degree ameliorate the effect of a shortage of one of the others?

The discovery by Ellis and Zmachinsky (1937 and subsequent experiments) that a high level of riboflavin intake increases the ability to withstand a deprivation of thiamin, may afford an affirmative answer to this last question; or may be interpreted upon the mass-action principle with the hypothesis of a common dissociation-

\* The nutritional chemistry of the nicotinic acid factor is outlined in the next chapter.

† The references and suggested readings listed at the end of the chapter will suffice to *start* the reader who wishes to go further; while for literature later than 1940 one will naturally consult the *Annual Review of Biochemistry* and the abstract journals.

product inasmuch as thiamin and riboflavin both contain the pyrimidine nucleus.

It is clear that riboflavin is a very important factor in the body's general tissue condition or internal environment; but not yet entirely clear in how many ways riboflavin functions.

### Quantitative Measurement and Expression of Riboflavin Values

According to present knowledge and indications, riboflavin values of foods may be considered as due to the one substance whose chemical nature has been given in the first section of this chapter; and, within the limits of normal nutrition at least, the nutritional availability and efficacy of riboflavin is essentially the same whether taken in the free state or in the form or forms of combination in which it largely occurs in plant and animal tissues.

Riboflavin is now so readily available in pure form that all quantitative work on riboflavin values of foods should be controlled, standardized, and interpreted by parallel operations with the pure substance, and the results are best expressed in terms of weight of actual riboflavin. Micrograms (gamma) of riboflavin per 100 grams of food is the scale of expression now generally regarded as most convenient and will therefore be used in this book.

As in the case of thiamin, *in vitro* methods for the determination of riboflavin are being developed by several research workers and may soon be coming into general use.

Meanwhile quantitatively controlled feeding methods in which side-by-side experimental animals receive in some cases graded amounts of riboflavin and in other cases graded amounts of the food under investigation, have been the means of establishing the most reliable data on riboflavin contents of foods yet available and are still (1940) the most widely used means of finding the riboflavin contents of foods.

When all the details of the feeding method of Bourquin and Sherman (1931) with the further precautions added by Page (1932) are followed,\* it appears that what was formerly called a "Bourquin-Sherman unit of vitamin G" is about 2.5 micrograms of riboflavin (Bessey, 1938, confirmed by several other workers).

As in the case of thiamin, the "negative control" animals of the

\* Some investigators have encountered difficulty with this method through failure to follow its conditions with sufficient exactness. There has also sometimes been a mistaken assumption that working at higher rates of growth is *ipso facto* more accurate.



experimental set-up for determining the vitamin value of a food may be made to serve also to demonstrate the effects of the vitamin deficiency; but as the characteristic symptoms develop much more slowly in riboflavin deficiency, and as a thoroughly safe control involves a more elaborate and troublesome technique, it seems better to refer the more technically inclined reader to the original papers indicated in the preceding paragraph (and later papers on technique) than to give a full description of such feeding experiments here.

### Foods as Sources of Riboflavin

Riboflavin is formed in the growth of green plants whose leaves and stems seem to be richest in riboflavin when in their most succulent stage, the riboflavin values decreasing as these organs dry and wither.

TABLE 49. — RIBOFLAVIN IN TYPICAL FOODS: MICROGRAMS PER 100 GRAMS

| Food                                   | RANGE WITHIN WHICH AVERAGE<br>WILL PROBABLY BE FOUND |
|--|--|
| <i>Foods of Animal Origin</i>          |  |
| Beef muscle . . . . .                  | 180-260  |
| Pork muscle . . . . .                  | 225-255  |
| Kidney (of cattle and swine) . . . . . | 1700-2200  |
| Liver (of cattle and swine) . . . . .  | 1800-2600  |
| Milk . . . . .                         | 195-240  |
| Eggs . . . . .                         | 280-420  |
| Egg white . . . . .                    | 150-300  |
| Egg yolk . . . . .                     | 380-750  |
| <i>Grain Products</i>                  |  |
| Wheat, entire . . . . .                | 100-220  |
| Wheat germ . . . . .                   | 600-800  |
| <i>Vegetables and Fruits</i>           |  |
| Banana . . . . .                       | 45-80  |
| Broccoli . . . . .                     | 200-500  |
| Cabbage . . . . .                      | 65-135   |
| Carrots . . . . .                      | 75-125   |
| Kale . . . . .                         | 400-600  |
| Lettuce . . . . .                      | 100-240  |
| Orange or juice . . . . .              | 28-90  |
| Spinach . . . . .                      | 250-400  |
| Tomato . . . . .                       | 37-63  |
| Turnip . . . . .                       | 50-100   |

Mature seeds contain measurable and significant amounts of riboflavin, but are not as rich in it as in thiamin.

As noted earlier in the chapter, riboflavin is widely distributed in plant and animal products, except those from which it has been rejected by artificial refining processes. Illustrations of quantitative distribution among all the chief types of food are included in Table 49 herewith and data for many other articles of food are tabulated in the Appendix.

It will be seen that the refined mill products of seeds are about as much depleted of riboflavin as of thiamin.

Leaves are relatively richer in riboflavin than in thiamin. Fruits, roots, and tubers are significant sources of both, but not very rich sources of either.

Eggs, lean meats, and milk are relatively rich sources of riboflavin. Beef and pork muscle do not show any such marked difference in riboflavin as they do in thiamin content. Milk is normally the most important source of riboflavin in the dietary.

### **Riboflavin Requirements in Human Nutrition**

The suggested statements of minimum requirements proposed for public hearings announced through the Federal Register provided: 0.5 milligram of riboflavin per day for infants; 0.75 milligram for children; and 1.0 milligram for adults. These were designed to represent absolute needs rather than desirable allowances.

M. S. Rose suggested as a standard for family dietaries, 50 to 60 micrograms of riboflavin per 100 Calories of total food. This may be taken as providing about 50 per cent more than the minimum estimates of the preceding paragraph.

Stiebeling and Phipard (1939) allow as a basis for the interpretation of the adequacy of family food supplies: 1.8 milligrams of riboflavin per day for all adults, for girls 14 years and over, and for boys 11 years and over; 1.6 milligrams for boys 7 to 10, and girls 8 to 13 years of age; and 1.3 milligrams for all younger children. These latter allowances are no higher than seem certainly desirable, whatever the minimum estimate of actual need may be. For there is no reason to doubt, and good reason to believe, that the *very wide margin of beneficial increase* over absolute minimal need is as valid for human beings as for the experimental animals with which it is being investigated as described earlier in this chapter.

Thus there is definite scientific basis for the belief that an intake high enough to keep the body "saturated" is as important in the case of riboflavin as of ascorbic acid. In fact the scientific basis is stronger in the case of riboflavin for here we have the evidence of controlled experiments extending throughout the life cycle and into the second generation.

Only a limited amount of experimental evidence is yet available upon the relation of the riboflavin intake to body saturation in man. Hogan (1939) has suggested from his study of this evidence that a man should receive from 2 to 3 milligrams of riboflavin daily. This is somewhat higher than the January 1939 standard of Stiebeling and Phipard but no more so than is commensurate with the most recent advances of our knowledge. Sebrell *et al.* (1941) suggest about 3 milligrams.

There is objective evidence of an exceptionally comprehensive kind for a wide margin between minimal requirement and optimal requirement in the case of riboflavin.

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## CHAPTER XX

### NICOTINIC ACID AND THE PELLAGRA PROBLEM

THROUGH the formation of compounds which act as coenzymes, nicotinic acid functions, in a manner more or less analogous to thiamin and riboflavin, in the system of tissue catalysts involved in the intermediary and oxidation-reduction metabolism. For the reasons explained in the discussion of riboflavin in the preceding chapter, it seems too early to attempt to summarize the chemistry of this complex field with such conciseness as a book of this size would require. The present chapter is therefore devoted essentially to the pellagra problem.

The word *pellagra* literally signifies a rough-red skin. The dermatitis and an accompanying inflammation of the tongue are the most striking and characteristic symptoms of the disease; but often there are also digestive and nervous or psychotic disturbances. The disease was first described as an affliction of the poor in certain parts of Southern Europe which were suffering severe privations in the aftermath of the Napoleonic wars. It was recognized in the United States, chiefly in the mill villages of the South, about 1907, and for a time there was controversy as to whether it was a nutritional deficiency or an infectious disease.

#### Discovery of the Pellagra-Preventing Substance

Goldberger and his coworkers in the United States Public Health Service established the nature of pellagra as a nutritional deficiency disease by a long series of investigations of which the most famous was the prison-farm experiment. Convicts under sentence in a Southern State for what the Governor regarded as reasonably pardonable offenses were promised pardons if they would voluntarily eat, for a year or until distinct symptoms developed, the diet which Goldberger considered typical of the food supplies which he suspected of causing pellagra.

One of the convicts found this diet so inferior to the regular prison-farm fare that he returned to the latter. The others contin-

ued on the test diet under excellent conditions of sanitation, and in a few months symptoms of pellagra began to appear. In much less than the year agreed upon, the majority of the group had developed pellagra and all were pardoned.

Searching experimentally for the substance involved, Goldberger and his coworkers found in 1926 evidence of the existence of something by virtue of which yeast still had antipellagric value after its antineuritic value had been destroyed by heating at autoclave temperatures. This antipellagric substance, pending chemical identification, was designated the *pellagra-preventive* (P-P) factor. Late in 1937, Elvehjem and coworkers at the University of Wisconsin showed nicotinic acid and its amide to have the property of preventing and curing "blacktongue" in dogs, a condition analogous to pellagra. Very quickly then came reports of the cure of pellagra by nicotinic acid almost simultaneously in at least four clinics.

Thus Goldberger's pellagra-preventive factor is chemically identified as nicotinic acid.

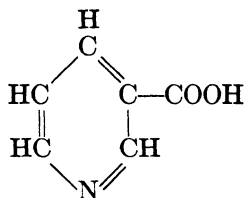


FIG. 34. — Nicotinic Acid

Sydenstricker, Sebrell, Cleckley, and Kruse (1940), in the paper cited in the preceding chapter, define their use of the term pellagra as follows: "The term pellagra is used arbitrarily to designate the syndrome of glossitis [inflammation of the tongue] and dermatitis, with or without psychic symptoms and diarrhea, specifically cured by nicotinic acid." Unfortunately, however, nicotinic acid alone does not enable the typical pellagrin to become a healthy person; for the food supplies which have been and still are responsible for the illness of most pellagrins are certainly often deficient in other things as well as nicotinic acid.

Hence the summary given beyond of kinds and amounts of foods which have been found effective in actual human experience is presumably of more value for present purposes than a table of nicotinic acid contents, or of blacktongue-preventing values, of foods.

### Other Factors Involved in the Pellagra Problem

As Ball (1939), Elvehjem (1939), and S. G. Smith (1940) among others have emphasized, at least three vitamins of the B group, (thiamin, riboflavin, and nicotinic acid) are collectively concerned in biological oxidations and any investigation of the rôle of one of these demands a consideration of the other two.

Adenylic acid, as a constituent of the pyridine dinucleotides apparently involved in pellagra, has been found by Spies, Bean, and Vilter (1940) to increase the efficacy of nicotinic acid in the treatment of some cases of pellagra, and also to be helpful in some cases of apparently analogous deficiency disease which did not respond to nicotinic acid treatment.

McLester, Spies, and others emphasize the general experience in pellagrous regions that while nicotinic acid cures the dermatitis and inflamed tongue which are the conditions to which the name pellagra specifically applies, and usually also alleviates the digestive and nervous disturbances, yet often (as explained above) the clinical pellagrin is not thereby rendered a fully healthy person. Multiple nutritional deficiencies seem, in the actual experience of American people, to be more frequent than deficiencies in nicotinic acid alone.

The typical diet of the poor pellagrin contains so little of anything else than milled cereals, sweets, and fats or fat meat that it is all too apt to induce other nutritional deficiencies at the same time with that of nicotinic acid.

Physicians dealing clinically with pellagra have repeatedly reported that while nicotinic acid cures the pellagra itself, many if not most clinical pellagrins need also thiamin or riboflavin or both. And Spies has reported a group of cases who after receiving all three of these vitamins were still not entirely well, but became so when given vitamin B<sub>6</sub> in addition.

Thus the nutritional problem of the typical poor pellagrin cannot be entirely solved by nicotinic acid alone.

What is needed in the pellagrous regions is a better food supply. Sebrell gives as the most important foods to add, "milk, liver, lean meats, fish, eggs, tomatoes, green peas, and a variety of green and leafy vegetables such as kale, mustard greens, turnip greens, and collards."

The deficient diet of the pellagrous regions is, he further writes,



“caused by the cultivation of a money crop instead of food and forage crops.” If even a small fraction of the time and land devoted to cotton were used for raising vegetables for the family and forage crops for a poultry flock and for a family cow, the health, and with it the earning power, of the people would undoubtedly be greatly improved.

The field studies of Sandels and Grady (1932) and of Stiebeling and Munsell (1932) both showed a very clear connection between food habits and pellagra incidence.

Bringing together the findings of the National Institute of Health and of the Bureau of Home Economics, it may be concluded that any one of the following contains a full day's quota of pellagra-preventive value:

A quart of milk or buttermilk;  
A pint of evaporated milk;  
One-third to one-half pound  
  of dried skimmed milk,  
  of lean meat,  
  of canned salmon,  
  of peanut meal, or  
  of wheat germ;  
One pound of fresh or canned collards,  
  of kale,  
  of green peas, or  
  of turnip greens; or  
Two to three pounds of tomatoes, fresh  
  or canned, or  
  of tomato juice.

These proportions based essentially upon work directly with people are probably more dependable than data of simple nicotinic acid or blacktongue-preventive values derived from experiments with dogs, for in the latter the values of milk and of vegetables are apt to be underestimated because dogs do not utilize these foods as well as do people.

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## CHAPTER XXI

### OTHER WATER-SOLUBLE VITAMINS AND SUBSTANCES OF RELATED INTEREST

IN THE terminology which the British biochemists introduced several years ago, water-soluble vitamins which came to light through differentiation of the original vitamin B were treated as members of the "B group" and distinguished by subscript numerals. On the other hand, when Szent-Gyorgy announced a differentiation of vitamin C, he called the postulated new substance vitamin P because of its supposed relation to the permeability of capillary walls. Each of these examples has been followed in some other cases, while in still others names have been suggested which avoid the use of the "overworked" word vitamin.

It is merely for convenience that substances of all these categories are included in this chapter. We are not attempting to answer any question as to whether a given substance "should be called a vitamin."

Because our knowledge of their nutritional significance is still so tentative, it seems best to give only very brief statements about the substances mentioned here, and to list references rather liberally at the end of the chapter for the convenience of such readers as may wish to go further.

#### Vitamins B<sub>3</sub>, B<sub>4</sub>, and B<sub>5</sub>

Vitamins B<sub>3</sub> and B<sub>5</sub> were postulated from observations upon pigeons, and not as nutritionally essential to mammalian species. Further work made it appear at least doubtful whether the effects attributed to vitamins B<sub>3</sub> and B<sub>5</sub> necessarily involved factors distinct from those known under other designations.

Vitamin B<sub>4</sub>, postulated as a nutritional essential for mammals, has attracted little attention except from the Wisconsin group, who in 1936 and 1939 have referred to a nutritional paralysis in rats as being probably "the same as that observed in vitamin B<sub>4</sub> deficiency."

### Vitamin B<sub>6</sub> (Pyridoxine)

Vitamin B<sub>6</sub> was the first designation of a then unidentified factor required for growth and for prevention of dermatitis in the rat. Because the dermatitis caused by its lack is, in the case of the rat, especially striking, vitamin B<sub>6</sub> was sometimes also called "the antidermatitis vitamin" and confused with the pellagra-preventive factor. Soon after the latter was identified as nicotinic acid, vitamin B<sub>6</sub> was shown to be 2-methyl-3-hydroxy-4, 5-di-(hydroxymethyl)-pyridine:

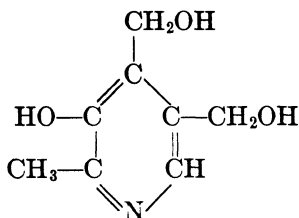


FIG. 35. — Pyridoxine (Vitamin B<sub>6</sub>).

and was given the name *pyridoxine* (adopted during the spring of 1940 by the American Institute of Nutrition, the American Society of Biological Chemists, and the Council of Pharmacy and Chemistry of the American Medical Association).

Spies, as we saw in the preceding chapter, reported indications of a vitamin B<sub>6</sub> deficiency disease occurring as a complication of pellagra; but Elvehjem, in a lecture summarizing the status of the members of the vitamin-B group before the American Society of Biological Chemists in March, 1940, designated this as a doubtful factor in human nutrition. Its rôle in human nutrition and its therapeutic possibilities are problems for further research.

The conspicuous relationship of this factor as observed in laboratory experimentation is to the prevention of the acrodynia-like dermatitis in rats. Birch (1938) reported evidence that both vitamin B<sub>6</sub> and a fatty acid or fat-soluble factor are concerned in the prevention of this dermatitis; and that the latter factor shows similarities to the essential fatty acid of Burr and Burr and to the fat-soluble antidermatitis factor of Hogan and Richardson.

Schneider, Ascham, Platz, and Steenbock (1939) reported a large number of foods, some of them fats and others presumably sources of vitamin B<sub>6</sub>, as being effective for the prevention of an

acrodynia-like condition. And more recent work from the Wisconsin laboratory (Schneider *et al.*, 1940) suggests that still another factor (not yet identified) may also be involved.

Salmon's preliminary findings seem to support the general position taken by Birch (1938) and favorably discussed by King (1939). At the 1940 meeting of the American Society of Biological Chemists, Salmon expressed the view that although vitamin B<sub>6</sub> and the essential fatty acids can to some extent alleviate the deficiency of each other, the presence of both in the diet is necessary for the normal nutrition of the rat.

### "Vitamin H"

The term vitamin H or factor H has been used in at least three ways: (1) by McCay for a factor nutritionally essential to trout; (2) by Booher for what now appears probably to have been the same substance as vitamin B<sub>6</sub>; and (3) by György for that which prevents the so-called "egg-white injury" and which seems from recent work (Eakin, McKinley, and Williams, 1940; du Vigneaud, Melville, György, and Rose, 1940) to be the factor studied by R. J. Williams and associates as *biotin*, a growth essential for yeasts.

### Vitamin M

Day, Langston, and coworkers discovered a nutritional deficiency disease of the monkey showing itself in the blood, as a cytopenia; and that a previously unknown factor is essential to the nutrition of the monkey for the prevention of this disease. This factor they named vitamin M.

### Vitamin P (Citrin)

This was postulated by Szent-Gyorgy as an additional factor involved along with vitamin C in the maintenance of normal conditions in the walls of the small blood vessels. There is not yet (1940) a clear consensus of opinion as to the existence and significance of this "permeability vitamin."

### Pantothenic Acid, "Filtrate Factor," Factor W, and the "anti-grey hair" Factor

Pantothenic acid was the name given by its discoverer, R. J. Williams, to the widely occurring substance now chemically identified as the beta-alanine condensation product of the substance

which may be called either alpha-hydroxy-beta, beta-dimethyl-gamma-butyrolactone or alpha, gamma-dihydroxy, beta, beta-dimethyl butyric acid.

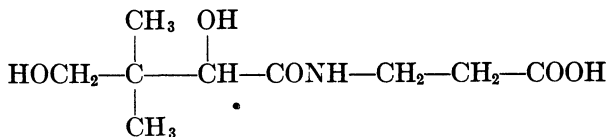


FIG. 36. — Pantothenic Acid ( $\alpha$ ,  $\gamma$ -dihydroxy- $\beta$ ,  $\beta$ -dimethylbutyryl- $\beta'$ -alanide)

According to the evidence available at time of writing, this seems to have been the substance studied earlier as the chick anti-dermatitis vitamin; and is apparently involved in (but seemingly does not account for the whole effect of) the so-called "filtrate factor" or "factor 2" of mammalian nutrition.

In the August 17, 1940, issue of the *Journal of the American Medical Association*, Spies, Williams, and Jukes with their respective coworkers united in reporting that their "observations indicate that pantothenic acid is essential to human nutrition and that its function is probably associated with that of riboflavin." They found that the injection of 200 micrograms of riboflavin per kilogram of body weight caused, under the conditions of their experiments, substantial increases in the concentrations of both riboflavin and pantothenic acid in the blood. Also the administration of calcium pantothenate was found by them to increase the concentration not only of pantothenic acid, but also of riboflavin in the blood of patients showing symptoms of riboflavin deficiency.

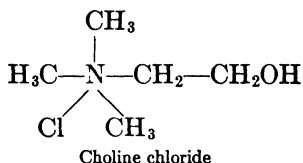
Factor W, postulated by the Wisconsin group as an additional nutrient required for the growth of the rat, and the factor described by Morgan and others as needed to prevent greying of the hair of black rats and loss of proper hair color in some other species, have not been identified (1940) with any previously known factor, nor as to their chemical natures.

### Grass Juice Factor

Independent and differently planned experiments in Wisconsin and at the University of California, indicate that the juices of fresh leaves contain some substance of nutritive value other than the nutrients chemically identified or above mentioned.

### Choline

While differing from most of the substances which we call vitamins in that its chemical nature and wide natural occurrence were well known many years before its nutritional importance became evident, *choline* is now often considered as a member of the "B-vitamin group."



It was noted by Best that choline prevented the abnormal accumulation of fat otherwise occurring in the liver of depancreatized dogs on certain types of diet; and later this same so-called *lipotropic* activity of choline was demonstrated in normal rats fed purified diets of high fat or cholesterol content.\* The diets in some of the early experiments were so distorted as to leave considerable doubt whether choline need be considered a factor in normal nutrition; but with increasingly successful efforts to eliminate choline from the experimental rations, it became possible to produce symptoms of choline deficiency without resort to grossly imbalanced diets. One of these symptoms is the disturbance of fat metabolism already indicated, which manifests itself in the development of so-called fatty livers. But, as Griffith has shown, other tissues may be affected as well. Thus he found that on diets still more rigorously freed of choline than is necessary to induce fatty livers, young rats within a very short time showed "hemorrhagic enlargement and degeneration of the kidneys, a regression of the thymus, and an enlargement of the spleen"; and he suggested that choline is essential for the maintenance of the normal structure of tissues as well as for its lipotropic action.

These observations become very suggestive when it is recalled that choline is a component of some of the phospholipids which, as we have already seen, may have both structural and metabolic rôles. The effect of choline in preventing the accumulation of fat in the liver was early interpreted as indicating that it facilitates lecithin synthesis, which, according to one theory, is an important

\* This aspect of choline metabolism has been extensively reviewed by Best and Ridout (1939).



step in fat metabolism. Direct evidence in support of this view was obtained by Perlman and Chaikoff who, using radioactive phosphorus as a tracer, showed that the rate of turnover of liver phospholipids is markedly accelerated by the administration of choline.

In ways not yet altogether clear, choline seems to be interrelated in its action with the sulfur-containing amino acids; for the choline-deficiency symptoms are said to be aggravated by cystine-rich diet and alleviated by liberal intake of methionine.

It appears also that choline may serve as a methylating agent in body processes. Thus it was found by du Vigneaud and his associates that homocystine (the next higher homolog of cystine) failed to replace methionine in a diet rigorously freed of choline; but that it became a nutritionally satisfactory substitute for methionine when choline was also supplied. Their suggested explanation was that a methyl group may be transferred from the nitrogen of choline to the sulfur of homocysteine, converting the latter to methionine. The reverse transfer was also demonstrated by the finding, after deuteromethionine (methionine containing deuterium in the methyl groups) had been fed, of a high percentage of deuterium in the body of rats maintained on a methionine-choline-free basal diet. This observation may afford at least a partial explanation of the suggested interrelationship of choline and the sulfur-containing amino acids.

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## CHAPTER XXII

### VITAMIN A AND ITS PRECURSORS

#### Chemical Structures and Interrelationships

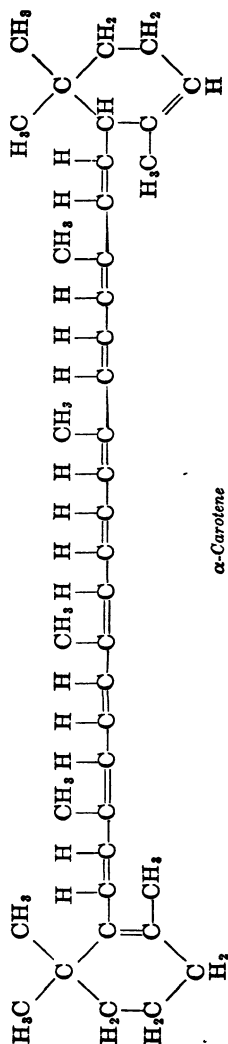
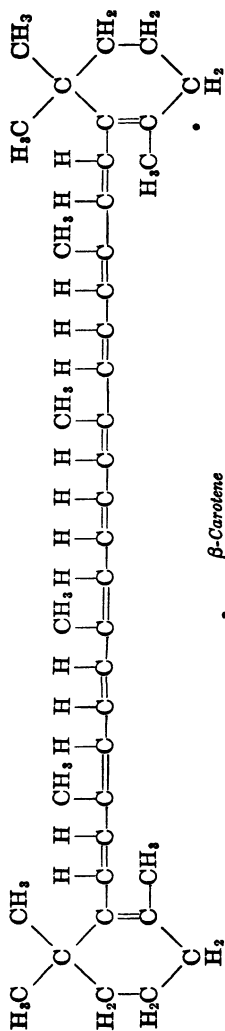
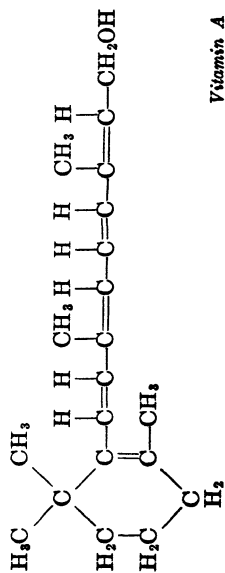
VITAMIN A itself is a colorless, fat-soluble substance, — or more strictly speaking, a pair of such substances, — occurring in relative abundance in the fats of milk, eggs, livers, and fish. It is found in especially high concentration in fish liver oils; and it now appears that there are two closely related substances, the one predominating in salt-water fish livers, being called vitamin A<sub>1</sub>, and that predominating in fresh-water fish livers, being called vitamin A<sub>2</sub>. It is convenient and scientifically admissible to continue to use the term vitamin A as a collective singular to cover these two substances functioning essentially as if they were one.

There is, however, another complication which does require recognition in our mode of speaking.

At least four substances (alpha-, beta-, and gamma-carotene, and cryptoxanthin) are known to be precursors of vitamin A. They are readily hydrolyzed in the body, chiefly in the liver, yielding vitamin A. Hence the presence of any of these precursors in the food adds to its *vitamin A value*, though not to its vitamin A content. The fish liver oils contain vitamin A and practically none of its precursors. Foods generally, however, owe their vitamin A values partly or wholly to the precursors which they contain.

Green leaves have a high vitamin A value, but no vitamin A content: the value is wholly due to the presence of the precursors. This is believed to be the case with the vitamin A values of all plant organs. Animal organs often contain both vitamin A and some unchanged precursor, usually carotene; and this is true of milk and eggs also.

The accepted structural formulae for vitamin A and its four known precursors are shown on pages 403 and 404. It should be noted, however, that the discovery of vitamin A<sub>2</sub> has thrown some of the details of the molecular structure into debate. (See, for instance, Embree and Shantz, 1940.)





C1=CC(=C(C=C1)C(=O)O)C2=CC=CC=C2C3=CC=CC=C3C4=CC=CC=C4C5=CC=CC=C5C6=CC=CC=C6C7=CC=CC=C7C8=CC=CC=C8C9=CC=CC=C9C10=CC=CC=C10C11=CC=CC=C11C12=CC=CC=C12C13=CC=CC=C13C14=CC=CC=C14C15=CC=CC=C15C16=CC=CC=C16C17=CC=CC=C17C18=CC=CC=C18C19=CC=CC=C19C20=CC=CC=C20C21=CC=CC=C21C22=CC=CC=C22C23=CC=CC=C23C24=CC=CC=C24C25=CC=CC=C25C26=CC=CC=C26C27=CC=CC=C27C28=CC=CC=C28C29=CC=CC=C29C30=CC=CC=C30C31=CC=CC=C31C32=CC=CC=C32C33=CC=CC=C33C34=CC=CC=C34C35=CC=CC=C35C36=CC=CC=C36C37=CC=CC=C37C38=CC=CC=C38C39=CC=CC=C39C40=CC=CC=C40C41=CC=CC=C41C42=CC=CC=C42C43=CC=CC=C43C44=CC=CC=C44C45=CC=CC=C45C46=CC=CC=C46C47=CC=CC=C47C48=CC=CC=C48C49=CC=CC=C49C50=CC=CC=C50C51=CC=CC=C51C52=CC=CC=C52C53=CC=CC=C53C54=CC=CC=C54C55=CC=CC=C55C56=CC=CC=C56C57=CC=CC=C57C58=CC=CC=C58C59=CC=CC=C59C60=CC=CC=C60C61=CC=CC=C61C62=CC=CC=C62C63=CC=CC=C63C64=CC=CC=C64C65=CC=CC=C65C66=CC=CC=C66C67=CC=CC=C67C68=CC=CC=C68C69=CC=CC=C69C70=CC=CC=C70C71=CC=CC=C71C72=CC=CC=C72C73=CC=CC=C73C74=CC=CC=C74C75=CC=CC=C75C76=CC=CC=C76C77=CC=CC=C77C78=CC=CC=C78C79=CC=CC=C79C80=CC=CC=C80C81=CC=CC=C81C82=CC=CC=C82C83=CC=CC=C83C84=CC=CC=C84C85=CC=CC=C85C86=CC=CC=C86C87=CC=CC=C87C88=CC=CC=C88C89=CC=CC=C89C90=CC=CC=C90C91=CC=CC=C91C92=CC=CC=C92C93=CC=CC=C93C94=CC=CC=C94C95=CC=CC=C95C96=CC=CC=C96C97=CC=CC=C97C98=CC=CC=C98C99=CC=CC=C99C100=CC=CC=C100C101=CC=CC=C101C102=CC=CC=C102C103=CC=CC=C103C104=CC=CC=C104C105=CC=CC=C105C106=CC=CC=C106C107=CC=CC=C107C108=CC=CC=C108C109=CC=CC=C109C110=CC=CC=C110C111=CC=CC=C111C112=CC=CC=C112C113=CC=CC=C113C114=CC=CC=C114C115=CC=CC=C115C116=CC=CC=C116C117=CC=CC=C117C118=CC=CC=C118C119=CC=CC=C119C120=CC=CC=C120C121=CC=CC=C121C122=CC=CC=C122C123=CC=CC=C123C124=CC=CC=C124C125=CC=CC=C125C126=CC=CC=C126C127=CC=CC=C127C128=CC=CC=C128C129=CC=CC=C129C130=CC=CC=C130C131=CC=CC=C131C132=CC=CC=C132C133=CC=CC=C133C134=CC=CC=C134C135=CC=CC=C135C136=CC=CC=C136C137=CC=CC=C137C138=CC=CC=C138C139=CC=CC=C139C140=CC=CC=C140C141=CC=CC=C141C142=CC=CC=C142C143=CC=CC=C143C144=CC=CC=C144C145=CC=CC=C145C146=CC=CC=C146C147=CC=CC=C147C148=CC=CC=C148C149=CC=CC=C149C150=CC=CC=C150C151=CC=CC=C151C152=CC=CC=C152C153=CC=CC=C153C154=CC=CC=C154C155=CC=CC=C155C156=CC=CC=C156C157=CC=CC=C157C158=CC=CC=C158C159=CC=CC=C159C160=CC=CC=C160C161=CC=CC=C161C162=CC=CC=C162C163=CC=CC=C163C164=CC=CC=C164C165=CC=CC=C165C166=CC=CC=C166C167=CC=CC=C167C168=CC=CC=C168C169=CC=CC=C169C170=CC=CC=C170C171=CC=CC=C171C172=CC=CC=C172C173=CC=CC=C173C174=CC=CC=C174C175=CC=CC=C175C176=CC=CC=C176C177=CC=CC=C177C178=CC=CC=C178C179=CC=CC=C179C180=CC=CC=C180C181=CC=CC=C181C182=CC=CC=C182C183=CC=CC=C183C184=CC=CC=C184C185=CC=CC=C185C186=CC=CC=C186C187=CC=CC=C187C188=CC=CC=C188C189=CC=CC=C189C190=CC=CC=C190C191=CC=CC=C191C192=CC=CC=C192C193=CC=CC=C193C194=CC=CC=C194C195=CC=CC=C195C196=CC=CC=C196C197=CC=CC=C197C198=CC=CC=C198C199=CC=CC=C199C200=CC=CC=C200C201=CC=CC=C201C202=CC=CC=C202C203=CC=CC=C203C204=CC=CC=C204C205=CC=CC=C205C206=CC=CC=C206C207=CC=CC=C207C208=CC=CC=C208C209=CC=CC=C209C210=CC=CC=C210C211=CC=CC=C211C212=CC=CC=C212C213=CC=CC=C213C214=CC=CC=C214C215=CC=CC=C215C216=CC=CC=C216C217=CC=CC=C217C218=CC=CC=C218C219=CC=CC=C219C220=CC=CC=C220C221=CC=CC=C221C222=CC=CC=C222C223=CC=CC=C223C224=CC=CC=C224C225=CC=CC=C225C226=CC=CC=C226C227=CC=CC=C227C228=CC=CC=C228C229=CC=CC=C229C230=CC=CC=C230C231=CC=CC=C231C232=CC=CC=C232C233=CC=CC=C233C234=CC=CC=C234C235=CC=CC=C235C236=CC=CC=C236C237=CC=CC=C237C238=CC=CC=C238C239=CC=CC=C239C240=CC=CC=C240C241=CC=CC=C241C242=CC=CC=C242C243=CC=CC=C243C244=CC=CC=C244C245=CC=CC=C245C246=CC=CC=C246C247=CC=CC=C247C248=CC=CC=C248C249=CC=CC=C249C250=CC=CC=C250C251=CC=CC=C251C252=CC=CC=C252C253=CC=CC=C253C254=CC=CC=C254C255=CC=CC=C255C256=CC=CC=C256C257=CC=CC=C257C258=CC=CC=C258C259=CC=CC=C259C260=CC=CC=C260C261=CC=CC=C261C262=CC=CC=C262C263=CC=CC=C263C264=CC=CC=C264C265=CC=CC=C265C266=CC=CC=C266C267=CC=CC=C267C268=CC=CC=C268C269=CC=CC=C269C270=CC=CC=C270C271=CC=CC=C271C272=CC=CC=C272C273=CC=CC=C273C274=CC=CC=C274C275=CC=CC=C275C276=CC=CC=C276C277=CC=CC=C277C278=CC=CC=C278C279=CC=CC=C279C280=CC=CC=C280C281=CC=CC=C281C282=CC=CC=C282C283=CC=CC=C283C284=CC=CC=C284C285=CC=CC=C285C286=CC=CC=C286C287=CC=CC=C287C288=CC=CC=C288C289=CC=CC=C289C290=CC=CC=C290C291=CC=CC=C291C292=CC=CC=C292C293=CC=CC=C293C294=CC=CC=C294C295=CC=CC=C295C296=CC=CC=C296C297=CC=CC=C297C298=CC=CC=C298C299=CC=CC=C299C300=CC=CC=C300C301=CC=CC=C301C302=CC=CC=C302C303=CC=CC=C303C304=CC=CC=C304C305=CC=CC=C305C306=CC=CC=C306C307=CC=CC=C307C308=CC=CC=C308C309=CC=CC=C309C310=CC=CC=C310C311=CC=CC=C311C312=CC=CC=C312C313=CC=CC=C313C314=CC=CC=C314C315=CC=CC=C315C316=CC=CC=C316C317=CC=CC=C317C318=CC=CC=C318C319=CC=CC=C319C320=CC=CC=C320C321=CC=CC=C321C322=CC=CC=C322C323=CC=CC=C323C324=CC=CC=C324C325=CC=CC=C325C326=CC=CC=C326C327=CC=CC=C327C328=CC=CC=C328C329=CC=CC=C329C330=CC=CC=C330C331=CC=CC=C331C332=CC=CC=C332C333=CC=CC=C333C334=CC=CC=C334C335=CC=CC=C335C336=CC=CC=C336C337=CC=CC=C337C338=CC=CC=C338C339=CC=CC=C339C340=CC=CC=C340C341=CC=CC=C341C342=CC=CC=C342C343=CC=CC=C343C344=CC=CC=C344C345=CC=CC=C345C346=CC=CC=C346C347=CC=CC=C347C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**Cryptoxanthin**

A molecule of beta-carotene,  $C_{40}H_{56}$ , yields two molecules of vitamin A,  $C_{20}H_{30}O$ . This carotene molecule is symmetrical and the point of hydrolysis is a double bond at the center of the molecule, each half taking on *both* the H and the OH of a molecule of water. *Beta-carotene* thus yields very slightly over its own weight of vitamin A.

The other precursors also hydrolyze at a double bond, but as their molecules are not symmetrical each of them yields only one molecule of vitamin A. Hence each of *these other precursors* has only one half as much vitamin A value as has beta-carotene.

### The Nutritional Chemistry of Vitamin A

It is of more than historical interest that the existence of what we now call vitamin A was discovered through experiments (made independently and almost simultaneously by McCollum and Davis and by Osborne and Mendel, in 1913) in which it was found that young experimental animals on diets good in all other respects would continue to grow and thrive or would cease growing and sicken, according as the sole fat in their food mixtures was butterfat or lard. And further work soon showed that egg fat and cod liver oil resemble butterfat in this respect while most commercial fats and oils are more like lard. The property by virtue of which the maternal organism concentrates vitamin A in the material (milk or egg) provided for the offspring seems to have a high *survival value* for both mammalian and avian species, which suggests that the substance serves fundamentally important functions and may often be a determining factor in the fate of the individual or the family.

*The importance of vitamin A to growth and development* is illustrated in the experiments and observations, just mentioned, through which the existence of this factor was discovered. Its *relations to the eyes* are also of great importance. Vitamin A is directly involved in the chemical changes upon which vision depends; and of late it has been the general view (McCollum *et al.*, 1939, p. 309) that a diminished efficiency of adaptation of vision to a changed intensity of light (dysadaptation, hemeralopia, nightblindness) will be the earliest observable effect of a shortage of vitamin A in the case of an otherwise normal person. This relationship of vitamin A to vision is effectively used by Gove Hambidge as an introduction to his summary of *Food and Life*.\* As the vitamin A which thus func-

\* Pages 3-4 of the 1939 Yearbook of the U. S. Department of Agriculture.

tions in vision is doubtless brought to the retina by the blood, a lowered concentration of this vitamin in the blood might logically be expected to accompany or precede the lowering of visual efficiency, and at the time this is written the evidence is somewhat conflicting as to whether the determination of the vitamin A content of the blood or the measurement of the light-adaptation (dark-adaptation) of vision is the more consistently and delicately responsive to differences of intake of vitamin A values.

### Vitamin A and Vision

From the viewpoint of the chemistry of nutrition, the outstanding relationships of vitamin A to vision are (1) that vitamin A is essential to the regeneration of visual purple, the bleaching of which in the retina is one of the chemical reactions involved in vision; and (2) as a consequence it follows that the ability to see in a dim light, or to adapt to a changed intensity of light, is very directly related to the abundance and immediate availability of the supply of vitamin A. The mechanism of vision is also two-fold: that of the rods and that of the cones of the (diagrammatic) physical structure of the retina.

Jeans and coworkers (1936, 1937), using visual adaptation tests, found seriously frequent evidences of shortage of vitamin A among school children, especially in low-income families. Subsequently much attention has been given to the improvement of instruments for use in such tests, to the critical study of details of technique and interpretation, and to the extension of this general method both in surveys of populations as to their nutritional status and in laboratory research upon the quantitative problems of vitamin A requirement in human nutrition. (See many references at the end of the chapter.)

Whether measurement of visual adaptation or of the concentration of vitamin A in the blood will prove the more valuable criterion is a research question, the answer to which will doubtless depend both upon the development of techniques and upon fuller and more critical knowledge of the nutritional significance of vitamin A. It is probable, for instance, that a *real* shortage of vitamin A in the body will inevitably impair the efficiency of visual adaptation; but also that such impairment may sometimes result from other causes. And because bodily storage may be such a large and imperfectly understood factor, the recent dietary and therapeutic history may

not always permit a sound judgment as to whether a real shortage exists.

### **Medically Diagnosed Shortages and Massive Remedial Doses**

Getz, Hildebrand, and Finn (1939) reported that about one tenth of the apparently healthy people, and fully one half of the tuberculous, examined by them showed indications of shortage of vitamin A. Halibut liver oil with its very high vitamin A content may be used as a means of giving the body large allowances quickly as a remedial measure. Therapeutic dosage up to 200,000 units per day has been reported. Just how well such very large intakes are utilized is not yet known and may vary widely with individuals.

In this connection it may be noted that, in visual adaptation work, Wald found massive doses less efficient in stocking the body than might have been expected from the results of steady feeding of good diets, and Hecht found that massive doses, given to people whose bodily stores had been drastically depleted, did not quickly restore them to their original efficiency of visual adaptation.

### **Vitamin A and Metaplasia**

*The relation of vitamin A to mucous membranes* is a fact of very far-reaching significance. It has been found to be important in the eyes, in certain secreting glands, in the respiratory system, the alimentary canal, and the urinary tract; and it is readily conceivable that other such relationships remain to be discovered.

Mori, Bloch, and Blegvad independently described diseased conditions of children which in the light of all evidence now available seem to have been due primarily to shortage of vitamin A. While xerophthalmia was the symptom which called attention to these cases, it was noted that there was also a high incidence of respiratory disease which was probably primarily due to the weakening of the respiratory system by the same dietary deficiency. Blegvad states that the deaths which occurred among the children who showed the eye trouble were due in most cases to respiratory disease. See also the immediately following section and the readings suggested at the end of the chapter.

### **Experimental Deficiency in Laboratory Animals**

In experiments with laboratory animals the most readily observable sign of vitamin A deficiency is usually the development of

an ophthalmia (xerophthalmia, keratomalacia, or conjunctivitis). This was first recorded by Osborne and Mendel in 1913 and has occurred so regularly (at least in the experience of most observers) as to be regarded as a fairly characteristic result of a lack of vitamin A. But it is only one of several results, and frequently not the most important. For further studies have shown that vitamin A deficiency results also in an increased incidence: of respiratory disease; of skin, ear, and sinus infections; of inflammations and infections of the alimentary tract; and even of renal calculi (Osborne and Mendel, 1917). Doubtless the underlying cause of the weakness which may show itself in so many ways is the histological change which Wolbach, Howe, and Church described in 1929 as follows: "The specific effect of the absence of fat-soluble vitamin A in albino rats, guineapigs, and humans is found in epithelial tissues. This effect is the substitution of stratified keratinizing epithelium for the normal epithelium in various parts of the respiratory tract, alimentary tract, eyes and paraocular glands, and the genito-urinary tract." See also Wolbach and Howe (1928), Bessey and Wolbach (1939), and other references at the end of the chapter.

The higher incidence of infection observed among individuals on diets of low vitamin A value may be largely due to the fact that the displacement of normal by squamous epithelial tissue implies not only a loss of local secretion but also a loss of the *cilia* which normally act with the secretion in cleansing the surfaces of the respiratory tract by expulsion of foreign particles. Plainly the normal surface thus tends to protect the respiratory system both from bacteria and from miscellaneous particles which might cause mechanical injury and so increase the danger of the establishment of infection by such bacteria as may not be expelled. It has also been suggested that the local roughness and stickiness which accompanies the breaking-up of the normal tissue (when it is first being replaced by the squamous) may also increase the chances of lodgment of bacteria which would normally have been expelled.

Thus even our present incomplete knowledge shows more than one way in which shortage of vitamin A may increase the dangers of infectious disease. Whether it specifically increases *susceptibility*, in the technical sense in which bacteriologists and pathologists are apt to use this term, we do not know. As yet emphasis tends to be laid upon the characteristic metaplasia of epithelial tissue which many investigators have observed to result from deficiency of

vitamin A and which structurally impairs what the *Journal of the American Medical Association* has called "the body's first line of defense." This metaplasia of the epithelium has been observed in many parts of the body and in every species which has been thoroughly studied. It probably underlies the external eye symptoms as well as the other ills above mentioned. In Tilden and Miller's experiments upon monkeys, it resulted in injuries to the digestive tract which were often fatal before the development of a conspicuous degree of ophthalmia.

That the influence of vitamin A, unquestionably important at the "first line of defense" against infections, extends also beyond this is shown by several investigations (among others those of Boynton and Bradford, 1931; of Lassen, 1931, 1932; and of McClung and Winters, 1932) in which infective agents have been *injected* under experimental control, and it has been found that the animals more liberally supplied with vitamin A survived better.

In order to avoid confusion, it should be kept in mind that there are wide differences in attitude among those presumably competent to discuss the relation of vitamin A to infection; and that these differences are to a considerable extent explained by the fact that authorities vary in their modes of approach to the problem. Those who have sought to connect vitamin A with the immunological mechanism as commonly conceived have reported negatively. Those whose approach is somewhat broader but who still concentrate upon the search for clearly direct, primary relations, tend to report either negatively, or inconclusively. But on the more comprehensive (if technically less precise) question, there is much positive evidence that the level of intake of vitamin A does (whether directly or indirectly, primarily or secondarily) influence the frequency, or severity, or duration, of infectious disease, — not specifically of any one infection alone, and also not equally of all infections.

Both age and previous feeding may influence the results of a vitamin-A-poor diet.

In a typical series of observations, three fourths of the rats placed upon vitamin-A-free food at the age of about one month (end of infancy) developed the characteristic ophthalmia before death; but it developed in only about one fourth of the rats that were from two to nine months old when subjected to the same dietary deficiency. Thus it is plain that, with all other conditions

uniform, the older animal is distinctly less likely to develop the ophthalmia even though he dies from the vitamin-A deficiency.

The older rats, however, proved more susceptible than the younger ones to lung infection, the frequency of which, in our experience as in that of Steenbock and other investigators, is increased by diet deficient in vitamin A. In one series of experiments (Sherman and Storms, 1925), the lungs of the rats that had been transferred to the vitamin-A-free diet at from four weeks to four months of age appeared normal at autopsy in nearly all cases, while nearly half the rats that were six or nine months old when subjected to the same dietary deficiency developed lung trouble.

Space is not available here for a full discussion of the physiology and pathology of vitamin A deficiency. The references at the end of the chapter will serve to put those readers who so desire into touch with the original literature and with review articles on this more medical side of the subject. Also, concise summaries from a more physiological viewpoint than that of the present text may be found in Rose's *Foundations of Nutrition* and in Sherman and Lanford's *Essentials of Nutrition*.

### **The Storage of Vitamin A in the Body**

That combination of physical and chemical properties which makes vitamin A capable of storage in the body, in quantities sufficient to meet its nutritional needs for significantly long times, is of great importance to nutritional wellbeing and health at all ages, and the clear recognition of the magnitude of this factor in the nutritional economy is highly essential to an adequate appreciation of the true significance of the intake of vitamin A values.

At some times, and in some quarters, there has been failure of due recognition of the true importance of the vitamin A value of the food supply, because of the very fact that bodily storage may protect from prompt penalty in periods of subsequent shortage.

Both for interpretation of experiments and for the everyday application of our new knowledge, it is well to keep in mind that the body does not quickly acquire nor quickly lose the full store of vitamin A which it is capable of taking on when habitually given a liberal intake. Cammack found this several years ago in experiments with moderately liberal daily intakes, and the same fact seems to have been responsible for some otherwise confusing ob-

servations after Wald's administration of massive doses. Conceivably it may also have a bearing upon lack of pronounced correlation between bodily stores of vitamin A and either blood values or light-adaptation data in the work of Steininger, Roberts, and Brenner (1939).

Diagrams and graphs illustrating the striking differences which result from different bodily stores of vitamin A resulting from different nutritional intakes have been given elsewhere (Sherman and Boynton, 1925, page 1648; Sherman and Cammack, 1926, pages 71 and 72; Sherman and Lanford, 1940, page 257).

Even at weaning time young animals may already have a considerable store of vitamin A in the body and thus be able to continue to grow for some time upon a diet carefully freed from vitamin A but adequate in all other respects. Young rats separated from their mothers at a uniform age of four weeks show very different growth curves and survival periods on the same experimental diet free from vitamin A, according to the vitamin A content of the family diet.

The body can also store vitamin A at later ages.

In one investigation (Sherman and Boynton, 1925) laboratory animals of known nutritional history were killed and dissected and carefully graded weighed amounts of their tissues were fed as sole sources of vitamin A to young experimental animals. In this way it was shown directly that there is an actual storage of vitamin A in certain tissues, and it became possible to study quantitatively the distribution of the stored vitamin in the body and also to determine directly the extent to which the vitamin A content of the body is influenced by the intake of this vitamin in the food. If adipose tissue and skin be ignored, at least nine tenths of the total vitamin A in the body of a well nourished adult rat is found in the liver. Weight for weight, kidney appeared about 40 times, lung more than 40 times, and liver between 200 and 400 times as rich in this vitamin as the muscle of the same animal in Boynton's experiments. Moderate differences in the vitamin A content of the food, such as are well within the range of variation likely to be encountered in human experience, resulted in large differences in concentration of this vitamin in the liver, and distinct differences in the amount of it found in lung tissue. That the vitamin content of the lung tissue is thus dependent upon the abundance of this vitamin in the food is of special interest because it has also been found that



the higher intake of the vitamin is accompanied by a lower incidence of lung disease.

Baumann, Riising, and Steenbock (1934) have also studied body storage as affected by nutritional intake and the distribution in the body. They assigned about 95 per cent of the body's vitamin A content to the liver. The *in vitro* method used by them did not reveal any vitamin A in the muscles. Further work on the barely demonstrable vitamin A of muscle is now (1940) in progress in the writer's laboratory.

In another study of storage of vitamin A in the body (Sherman and Cammack, 1926) the placing of animals upon diet devoid of vitamin A was preceded by the feeding for different lengths of time of a diet bountifully supplied with the vitamin. This resulted in very great prolongation of life upon the vitamin-A-free food, showing that large surpluses of vitamin A can be stored under these conditions also. The maximum storage of vitamin A in rats of different ages was closely approached by feeding a ration containing 4 per cent of cod liver oil, which supplied about 80 times as much vitamin A as appeared necessary for adequate nutrition. However, the store does not increase in arithmetical proportion to the richness of the diet in vitamin A, for apparently the animal uses the vitamin provided in the food more freely or less economically as the maximum store is approached. Feeding of this ration which promoted greater storage of vitamin A did not alter the age of maximum storage (six months in the rat). However, it was possible to build up as large a reserve in a younger animal as in a mature animal of less fortunate dietary history.

Since the storage of vitamin A in the body has been shown to be such an important factor, it becomes probable that the supposed lesser need for this vitamin by the adult than by the young is apparent rather than real, and is largely attributable to the fact that the adult has had more opportunity to provide himself with a bodily store of this vitamin which then carries him over periods of deficient intake.

### Demonstration and Measurement of Vitamin A Values

When a diet lacking vitamin A value but adequate in all other respects is given to a young growing rat, growth continues for a shorter or longer time according to the extent of the stores of this vitamin which the body already possesses, after which growth

ceases and there usually soon sets in a loss of body weight and a condition of decline in health. About when the body's reserves of vitamin A have been exhausted and growth ceases, a large proportion of the experimental animals begin to develop a characteristic disease of the eye. This usually begins with a swelling of the lids of one or both eyes or with indications that the eye is becoming unduly sensitive to light. Following this there commonly develops an inflamed and catarrhal condition of the conjunctivae, with a bloody or purulent discharge, the lids becoming scabby or sticky. This, with the swelling of the lids and the sensitiveness to light, sometimes results in the eye being found completely closed. If the eye condition is not treated, and the animal continues to live, the cornea may become affected and blindness result. On the other hand the eye disease, if not too far advanced, usually disappears quickly upon feeding the animal with any food of adequate vitamin A value.

Thus a demonstration of vitamin A value in a food may be made by feeding the food to be tested to a young rat which has recently ceased to grow and begun to develop eye trouble from vitamin A deficiency as just described. Under these conditions if the test food has significant vitamin A value it will usually induce resumption of growth and recovery from the eye disease.

And such testing of foods can be made quantitative by standardizing the conditions and measuring the rate of growth induced by the feeding of graded allowances of the test food and of a reference material of accurately known vitamin A value.

The Health Organization of the League of Nations supplies a pure beta-carotene of which 0.6 microgram has, by definition, one International unit of vitamin A value. The U. S. Pharmacopeia unit of vitamin A value is the same; but the Pharmacopeia organization provides a reference material, more convenient for ordinary use than carotene, in the form of a codliver oil whose value has been measured in comparison with the International Standard carotene.

Samples of this *standard reference codliver oil* may be purchased from the U. S. Pharmacopeia Office, 43rd Street and Woodland Avenue, Philadelphia.

Such standard reference material is to be fed side-by-side with the food or other material under examination in every experiment, test, or assay for the measurement of a vitamin A value.

The same test may be made both qualitative and quantitative by permitting development of the typical sore eye before the feeding

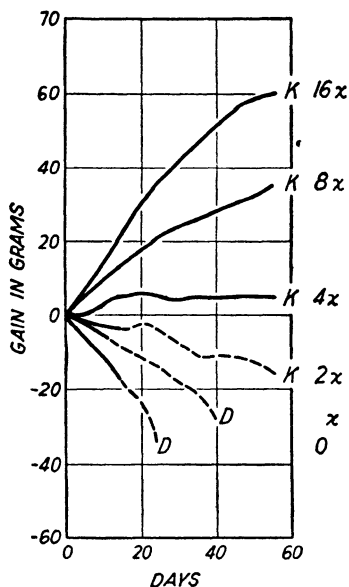


FIG. 37. — Average gain-curves of rats fed different amounts of vitamin A after having been depleted of their bodily stores (experiments of Dr. E. L. Batchelder). Relative amounts of vitamin A fed are shown at the ends of the curves. When all animals died before the end of the 8 weeks period (K), the curve is terminated at a point representing the average weight and age at death (D). (Courtesy of the *Journal of Biological Chemistry*.)

ing of the test food is begun; but if this involves serious illness in the test animal it is apt to render the results more variable, so that for quantitative work the test feeding is usually begun before the test animal becomes seriously ill, while at least one member of each litter or set of test animals is continued on the basal diet alone as a *negative control* in which the typical development of the vitamin A deficiency disease can be observed.

Quantitative measurements of the relative amounts of vitamin A in food can be made by means of sufficiently numerous and carefully controlled feeding experiments with rats which are amply provided with all other nutrients which they require.

The general plan (originally suggested by Drummond and Coward) is to start with normal young rats 21 to 29 days old, feeding them a basal diet\* good in all other respects but free from vitamin A. When any surplus store of vitamin which the body contained at the beginning has been depleted (or sufficiently diluted by the growth of the animal) the body weight ceases to increase and usually remains nearly stationary for a few days. At this time, if not before, each of the animals is placed in a separate cage and kept under controlled conditions. One (or more) of each litter continues to receive the basal ration only, until it dies from lack of vitamin A, thus serving as a negative control. Other ani-

\* For detailed descriptions of basal diets and techniques of testing, see Coward (1937), the American Chemical Society monograph on vitamins (Sherman and Smith, 1931), and the current *U. S. Pharmacopeia*.

mals are fed, in addition to the basal diet, systematically graded amounts of the food which is being tested.

Natural biological variability makes it probable that the results of individual experiments of this kind will vary more than should the data of chemical experiments conducted *in vitro*; but when sufficient numbers of animals are used and the results are averaged, the average weight curves show a fairly regular gradation according to the amount of vitamin A which the animal actually received.

The accompanying graph (Fig. 37) shows the results obtained

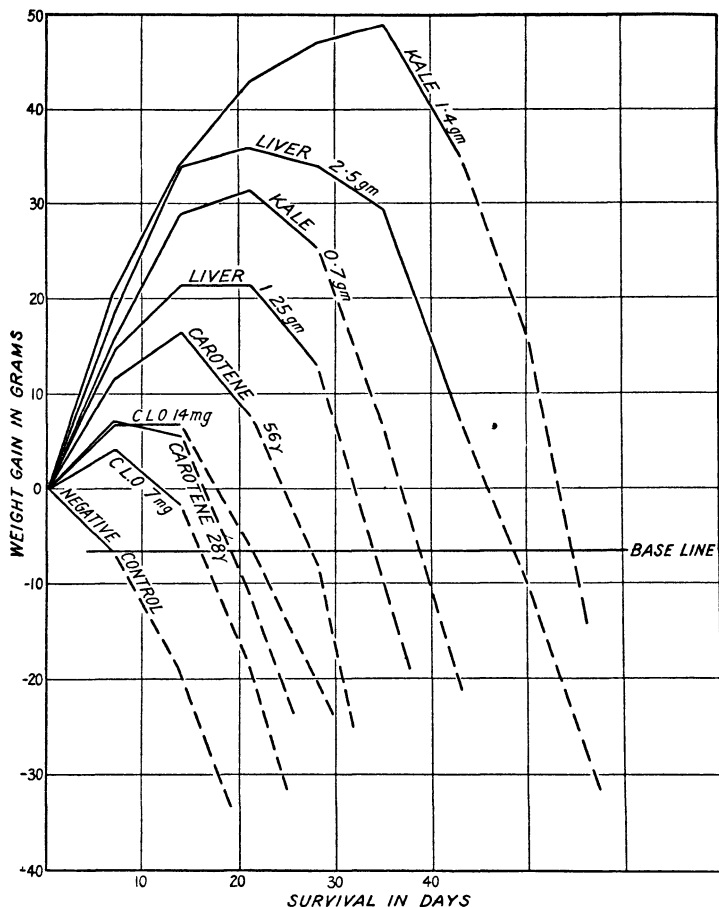


FIG. 38. — Average weight-curves of rats which had been depleted of bodily vitamin A in the same manner as those of Fig. 37, and to which single feedings of test material were then given. (From experiments by Dr. E. N. Todhunter.)

in negative controls and in test animals fed at several different levels of vitamin A intake (allowance of test food), each curve being the average result obtained with nine test animals. The starting point of these curves is the end of the *depletion period* and beginning of the *test period*.

In the experiments of Todhunter, vitamin A values were determined by a method of single feedings (Sherman and Todhunter, 1934). The typical results summarized in Fig. 38 are of interest in demonstrating the similarity of weight-curve response to equivalent vitamin A values whether in the form of the vitamin itself (as in cod liver oil) or its precursor (as in the case of carotene or kale) or a mixture of the two (as in liver); and also in showing the quantitative relationship of weight gain and survival period to the amount of the single feeding of any of these typical sources of vitamin A value. The result of this relationship is that the area, bounded above by the weight curve resulting from a given feeding and below by the negative control curve and the base line, is (under standardized conditions) quantitatively proportional to the vitamin A value of the weighed amount of test material used in the single feeding. This makes it possible to measure quantitatively the vitamin A values of materials which are only occasionally available, or of individual samples of fresh foods without subjecting them to the possibility of any decline in vitamin A value due to drying or storage.

### Vitamin A Values of Foods

The carotenes which constitute the chief precursors of the vitamin A of normal human nutrition, as well as of that of ordinary farm animals, are formed in plants. Typically the carotene content of a plant increases up to about the time of flowering, the accumulation of the carotene being superficially masked by the accumulation of chlorophyll. (A thriving condition usually involves abundant formation of carotene, and of chlorophyll.) The functional interrelationship of the carotenes and chlorophylls in the nutritional processes of the plant is still a problem of active research. The action of light does not seem to be strictly essential to the formation of carotene, for MacLeod and coworkers have observed an instance of increase of vitamin A value in a root (sweet-potato) on storage, and some small increases have been reported in etiolated seedlings; but, as shown by Drummond and Coward,

the pronounced development of vitamin A value in a seedling accompanies the appearance of green color, so that carotene and chlorophyll formation normally go on in a more or less parallel way in young plants and green leaves. The green leaves of food and forage crops and of pastures serve directly and indirectly to supply us with vitamin A values. The high vitamin A values of typical pasture grasses have been accurately measured by Woods and co-workers (1932, 1935). When we eat green leaves, we absorb (perhaps with varying degrees of completeness) the carotenes which they contain; and our bodies convert these carotenes (again, perhaps with varying degrees of completeness) into vitamin A which thus becomes available either for immediate nutritional needs or for storage in the body. And the carotenes of a wider range of green leaves are brought indirectly into the service of human nutrition through farm animals — particularly milch cows and laying hens. The cow and the hen render important services to human nutrition by bringing into the readily digestible and assimilable forms of milk and eggs a significant share of the nutritive values of leaves of coarser quality than we care to eat or which would exceed the quantity that we could readily digest. (Among agricultural experts there is a friendly rivalry between the poultrymen and the dairy men as to whether the hen or the cow is the more efficient servant of the human family in this extraction and transmission of nutritive values. In respect to vitamin A values, the hen at present appears the more efficient, while milk production exceeds egg production in efficiency with respect to energy value and to calcium. With respect to protein and riboflavin, quantitative comparisons of the efficiencies of dairy cows and laying hens are not at hand as this is written.)

In any broad view of the problem of food supply, the outstandingly important sources of vitamin A value are the green and yellow vegetables, eggs, milk, and such products of milk as contain a significant proportion of its fat (*e.g.*, butter, cream, ice-cream, and whole-milk or "cream" cheeses). There follows a brief discussion of these and other food sources of vitamin A as illustrated in Table 50.

The vitamin A values thus far reported for green leaf vegetables vary rather widely, even for the same species. This is doubtless attributable in part to actual variability of the materials and in part to laboratory errors due both to use of methods still in proc-

TABLE 50. — VITAMIN A VALUES OF TYPICAL FOODS: RANGE WITHIN WHICH THE AVERAGE MAY BE EXPECTED TO BE FOUND

| Food   | INTERNATIONAL UNITS<br>PER 100 GRAMS |
|--|--------------------------------------|
| Green leaves such as kale, spinach,<br>turnip greens ..... | 13,000-27,000                        |
| Peas, fresh young green .....                              | 1000-1300                            |
| Mature seeds generally .....                               | nearly negligible                    |
| Mill products and breadstuffs .....                        | negligible                           |
| Sugar and sweets .....                                     | none or negligible                   |
| Fruits: Apples .....                                       | 60-80                                |
| Bananas .....  | 160-400                              |
| Cantaloupes .....  | 400-2400                             |
| Oranges .....  | 50-400                               |
| Vegetables: Broccoli .....                                 | 3000-9000                            |
| Carrots .....  | 2200-4000                            |
| Potatoes .....   | 30-50                                |
| Sweetpotatoes .....  | 1500-3500                            |
| Tomatoes .....   | 500-2500                             |
| Turnips .....  | 10-20                                |
| Beef muscle .....  | 10-50                                |
| Milk .....   | 160-225                              |
| Butter .....   | 3500-5000                            |
| Eggs .....   | 1000-2000                            |

ess of quantitative development and to differences in the aptitude and experience of the laboratory workers.

The evidence now available on the green leaves used as food for man indicates that representative samples contain from 13,000 to 27,000 International units \* per 100 grams; but it should be emphasized that such high values are to be expected only in the thin green part of the leaf, and only in those leaves that are *fully* green. Leaves which are relatively colorless must be expected to be of low vitamin A value. Thus Kramer and coworkers found less than one-thirtieth as much vitamin A value in the white inner leaves of head lettuce as in the green outer leaves of the same plants. And the white inner leaves of tight-headed cabbages are of very much less vitamin A value than the green leaves of the loose-leaf cabbages, while the still greener leaves of the closely related kale have still higher vitamin A values.

\* As only International units (which are also the U.S.P. units) are used in this book, they may be referred to simply as units in this chapter and the one following.

Quinn, Burtis, and Milner (1927) demonstrated the correlation with greenness in plant tissues other than leaves when they found string beans and green peppers to be of high vitamin A value. Crist and Dye extended this to the case of green *versus* bleached asparagus tips.

The vitamin A values of other parts of plants are in general much lower; but in some cases vary widely with the extent to which carotene is transported to, and stored in, some organ other than the leaf. Thus most roots and tubers are of very low vitamin A value, while the carrot with its characteristically high carotene content shows regularly a high vitamin A value, and in sweetpotatoes the value is high but varies widely with the depth of yellow color. But not all yellow plant pigments are precursors of vitamin A, and the flesh of a root or fruit may perhaps show more yellow color than that of an apple or banana without necessarily having a higher vitamin A value. However, MacLeod has shown that in sweetpotatoes, and Smith has shown that in squash, the differences in depth of yellow color of the flesh are indicative of corresponding differences in carotene content and thus in vitamin A value.

As already noted, the leaves of forage plants and pasture grasses are of high vitamin A value. (The extent to which this value is conserved in hay depends largely upon the curing process.) The precursors obtained from such materials are largely transformed into actual vitamin A in the liver. The livers of meat animals are, therefore, apt to contain a relatively high concentration of vitamin A value due to the presence both of the precursor and of the vitamin formed therefrom, for both of which the liver serves to a considerable extent as a place of storage. But storage depends upon surplus. Hence liver is a variable source of vitamin A, though usually a fairly rich source *so far as it goes*. The latter phrase should be emphasized because liver is in its nature only a by-product of very limited significance from the viewpoint of the food supply as a whole. Increased appreciation of, and consumer demand for, liver cannot be met by increased production of liver as an independent food-crop. For each additional pound of liver produced, there must be produced many pounds of other meat; and the muscle meats are poor in vitamin A however the animal has been fed. The adipose tissues are an unreliable and usually unimportant source. Beef fat usually contains a little, and



lard or pork fat practically none. We do not know whether this is a true species difference or due to the fact that bees are fed more largely on grass or green fodder, while swine are fattened rapidly by intensive grain feeding. Grains (and therefore our cereals and breadstuffs) are of very low vitamin A value.

As milch cows may consume grains and grasses in quite different proportions, a question arises as to the extent of the resulting variations in the vitamin A values of milk. *In the long run* the nursing mother or the lactating animal is dependent upon her food for all the vitamin A value which she puts into her milk; but the storage capacity of the body for vitamin A permits a regulatory process by which the vitamin A value of the milk can be maintained during considerable periods of low intake by drawing upon the body stores accumulated in periods in which the intake was high. Thus the vitamin A value of the milk ultimately depends upon, but does not literally vary with and as, the vitamin A value of the food. In the experiments of Fraps and Treichler, the effect of the cow's body stores in keeping up the vitamin A value of her milk notwithstanding deficiency of vitamin A value in her feed was markedly significant for many more months than the ordinary winter period of dry feeding. In an analogous way, the hen uses her body store to regulate the vitamin A value of her eggs, though ultimately these body stores depend upon the vitamin A value of what she eats. In order to demonstrate clearly this dependence of the body upon its food, experimenters have in several cases artificially set up much more drastic differences than are likely to occur in farm practice, with the result that the scientific literature tends to give a very exaggerated impression of the extent to which the vitamin A values of milk and eggs vary.

The vitamin A values of sea foods originate much as do those of the land. Diatoms, small algae, and other tiny aquatic plants which have thus formed provitamin A may then be eaten by small crustacea, these by fish, and these in turn by larger fish like the cod. Thus the cod, through perhaps two or three or more intermediaries has obtained its vitamin A from sources ultimately analogous to those which supply the cow.

The U.S.P. standard for codliver oil calls for not less than 850 units *per gram*, and halibut liver oil is much richer in vitamin A (having, according to McCollum, about 20,000 units *per gram*), while percomorph oil is still richer.

### **Quantitative Requirements of Human Nutrition**

At present it appears probable that measurements of efficiency of adaptation of vision may be made, at least for people of all ages except early childhood, the most direct basis for estimates of minimal quantitative requirements. A project for such studies in the Federal Bureau of Home Economics is described on pages 227-229 of the 1939 Yearbook of the U. S. Department of Agriculture and a series of measurements have been reported by Booher, Callison, and Hewston (1939).

In this work five subjects, three women aged 30 to 40 and two men aged respectively 21 and 25 years, all "ostensibly healthy," served as subjects. When maintained on a basal diet planned to be good in other respects but to furnish less than 103 units of vitamin A per day, these subjects began to show clear evidence of diminished efficiency of adaptation after 16, 27, 29, 39, and 124 days, respectively.

When the vitamin A requirements of these five subjects, as judged by full efficiency of adaptation, was supplied by vitamin A itself the apparent need ranged from 25 to 55 units per kilogram of body weight.

When supplied in the form of carotene dissolved in oil, from 43 to 103 units of vitamin A value per kilogram of body weight were required.

Figured to requirements per 70 kilograms (average man) these would be:

In the form of vitamin A itself, 1750-3850 I.U. per day;

In the form of carotene, 3010-7210 I.U. per day.

Later experiments seem to show a better utilization of carotene in the form of vegetables than as given in solution in oil.

### **Distinction between Minimal and Optimal Standards**

The five subjects of Booher and coworkers whose depletion periods as we saw in the preceding section ranged from 16 to 124 days on the same vitamin A-deficient diet may well illustrate the wide differences of "vitamin A level" which undoubtedly exist among our "ostensibly healthy" people even in a given locality and economic group. Thus Gillett and Rice found that families of the same economic grouping in New York City spending practically the same amount per capita for food in the same market,

but according to their own preferences within their economic limitations, were actually consuming four or five times as much vitamin A value per person in some families as in others.

The higher levels of daily intake and of bodily storage constitute a sort of insurance, the value of which may or may not become clearly manifest in any given case, depending upon the unpredictable vicissitudes of individual lives. Mellanby has reported that increased allowances of vitamin A (or precursor) continue to diminish the dangers of infection up to levels of intake four times that required for normal nutrition in absence of exposure.

Full-life experiments with laboratory animals seem to afford the best indications yet available as to the long-time probabilities of people living under everyday conditions.

Batchelder found progressive improvement of nutritional well-being, as judged by entire life histories and the records of the offspring, upon successive increments of vitamin A intake up to about four times the minimal-adequate level. These experiments are now being extended by other workers in the Columbia laboratory with parallel studies of the life histories of the experimental animals and the bodily contents of vitamin A at different ages.

When it is possible to correlate the findings of further full-life experiments with those of blood studies like that of Steininger, Roberts, and Brenner (1939) we may be able to form clearer and more confident views as to the exact values of different margins of vitamin A intake above immediately demonstrable need. To this general suggestion we shall recur in Chapters XXVII and XXX.

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## CHAPTER XXIII

### THE VITAMINS D

#### Discovery of the Existence of an Antirachitic Factor

MELLANBY found in experiments with puppies that their skeletal development was influenced by some fat-soluble substance or substances in the food. He described this as a prevention of rickets by virtue of an antirachitic property of certain fats.

McCollum and his coworkers found that this was also true with rats, and that codliver oil still had a specific favorable influence upon skeletal development, or protected them from rickets, after its vitamin A value had been destroyed. This clearly indicated the existence of a fat-soluble factor other than vitamin A. It soon came to be called, interchangeably, vitamin D and antirachitic vitamin.

#### Chemical Structures of the Vitamins D

As more fully explained by Bills (1939) the term vitamin D is customarily applied to substances of the sterol group which have

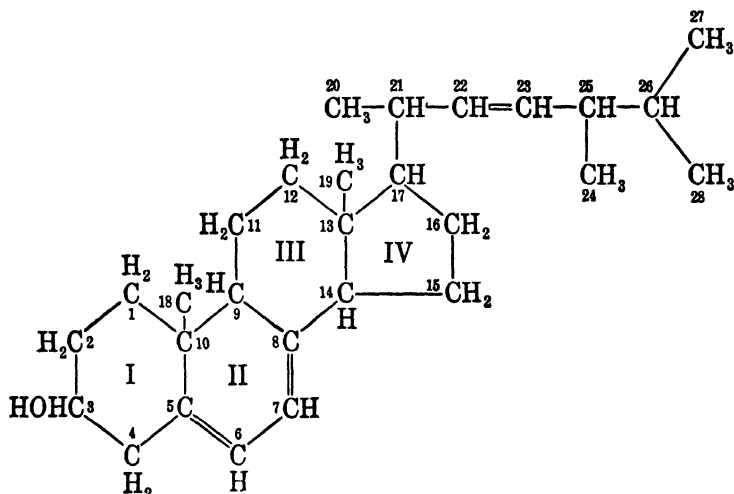


FIG. 39. — Ergosterol.

the antirachitic property. There are probably at least ten such substances, and five of them are (1940) fairly well defined as chemi-

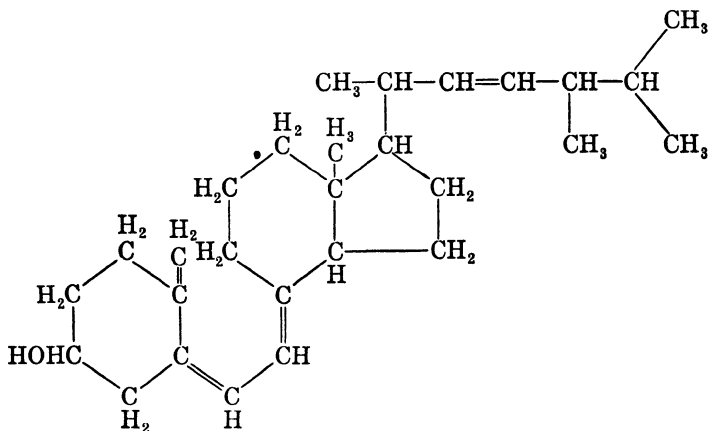


FIG. 40. — Vitamin D<sub>2</sub> (calciferol).

cal individuals; but only two of these are of outstanding importance.

The two important vitamins D are vitamin D<sub>2</sub> (activated ergosterol) and vitamin D<sub>3</sub> (activated 7-dehydrocholesterol).

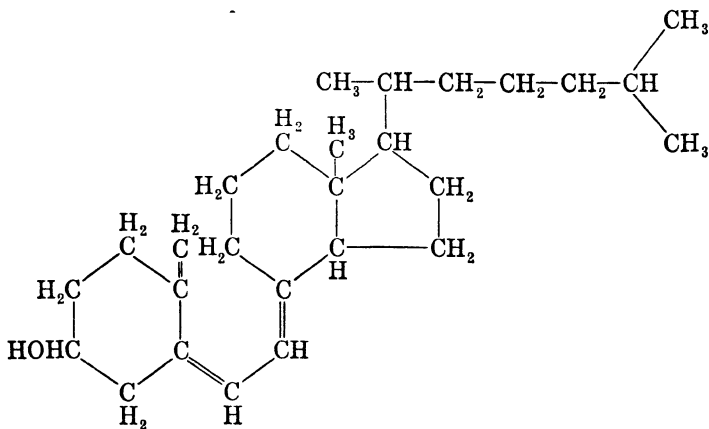


FIG. 41. — Vitamin D<sub>3</sub> (isolated from fish liver oil and also prepared by irradiation of 7-dehydrocholesterol).

Figures 39, 40, and 41 show the generally accepted structural formulas of ergosterol, vitamin D<sub>2</sub>, and vitamin D<sub>3</sub>, respectively.

In the formula for ergosterol, the carbon atoms are given their conventional position numbers. In terms of this formula, cholesterol ( $C_{27}H_{46}O$ ) differs from ergosterol in lacking the double bonds at  $C_7 : C_8$  and  $C_{22} : C_{23}$ , and in lacking the methyl group attached to  $C_{25}$  in ergosterol. On the other hand, 7-dehydrocholesterol has a double bond between carbons 7 and 8 but otherwise has the cholesterol structure just explained.

This 7-dehydrocholesterol has been shown to occur in the skin and is the chief "animal" precursor of vitamin  $D_3$ , just as ergosterol is the chief "plant" or "vegetable" precursor of vitamin  $D_2$ .

### Production of Vitamin D by Irradiation

In 1924 it was discovered by Hess and independently by Steenbock that various foods can be endowed with (or enriched in) anti-rachitic value by irradiation with certain wavelengths of ultra-violet light.

Ergosterol, concentrated industrially from yeast, was found to be a good starting material for such "activation" by irradiation. The particular vitamin D formed by activation of ergosterol is the one known as vitamin  $D_2$ . Its commercial forms include calciferol and viosterol.

Vitamin  $D_3$  formed by similar activation of 7-dehydrocholesterol is in some cases more effective nutritionally than vitamin  $D_2$ , and it has been identified with the principal natural vitamin D of fish liver oils (and presumably also of milk and eggs). Further activation studies of Bunker, Harris, and Mosher (1940) have also strengthened the evidence that 7-dehydrocholesterol is the substance which is activated, and vitamin  $D_3$  the form which is produced, upon irradiation of the skin.

There is still room for difference of expert opinion as to how strong a preference for the natural or animal form of vitamin D (vitamin  $D_3$ ) over the artificial form (vitamin  $D_2$ , calciferol, viosterol) is justified in the strict problem of the mere prevention of rickets in infants. In the broader view of child nutrition the fact that the natural form as obtained in milk and eggs, and in or from fish liver oils, is accompanied by vitamin A, and the further fact that the artificial form may not be wholly free from undesirable by-products, are significant practical reasons for preferring the natural or animal form.

Chicks and rats differ widely from each other in their responses

to vitamin D<sub>2</sub>: for the rat it is a relatively effective antirachitic, and the potency of its preparation is measured by tests with rats. International and U.S.P. units are therefore rat units. "Rat unit for rat unit," the natural form of vitamin D is enormously more effective in the nutrition of the chick than is the artificial form.

Bills emphasizes the fact that from a given provitamin only one form of vitamin D is produced by irradiation whatever the wavelength or other conditions. The conditions influence the by-products but the vitamin is always the same for the same precursor. He adds that processes in which electron bombardment is substituted for ultraviolet irradiation produce the same kind of vitamin.

### The Prevention of Rickets

According to Park's definition which has been generally accepted, rickets is a condition in which the mineral metabolism is disturbed in such a way that calcification of the growing bones does not take place normally. To guard against confusion, one must keep in mind in reading the literature of rickets that the term has often been given a narrower definition than Park gives it, older definitions turning more upon the histology of the bone tissue while Park's is expressed in terms of calcification in the developing bone, — an essentially chemical phenomenon.

The failure of normal calcification in the young bone is not usually to be attributed to any initial fault of the bone itself, for Shipley found that rachitic bones placed in normal blood serum began at once to calcify normally; and Kramer and Howland found in rickets a subnormal concentration of either calcium or phosphorus (or both) in the blood serum. Thus there are different types of rickets which may be classified according to the nature of the chemical deficiency in the blood.

*When the calcium content of the serum is normal but its phosphorus (phosphate ion) content is subnormal there results the so-called low-phosphorus rickets which corresponds most closely and completely to the classical histological descriptions of the disease, such as those of Schmorl. There is in such cases not only a characteristic pathological histology but also a tendency to overgrowth of the cartilaginous or osteoid tissue at the ends of the long bones and at the rib junctions. Because the retarded calcification prevents the bones from acquiring normal rigidity, the pressure of the body*

weight tends further to enlarge the ends of the bones. Together these abnormalities make a typical (and hitherto all too familiar) picture recognized in part by the obvious clinical signs of enlarged joints and the "rachitic rosary" and in part by means of the Roentgen ray photograph and the determination of inorganic phosphate in the blood serum; and confirmable at autopsy by histological examination. There has sometimes been a tendency to confine the term rickets or "true rickets" to this low-phosphorus type.

*When the phosphorus content of the serum is normal but its calcium content is subnormal* there results a similar gross abnormality but a somewhat different histology of the bones. This has sometimes been called a "rickets-like condition" or a "second type of rickets," but is now commonly designated as *low-calcium rickets*. Low-calcium rickets is often accompanied by tetany; and it seems not improbable that in such cases the trouble with the nerves as well as with the bones is attributable (at least in part) to the deficiency (subnormal concentration) of calcium in the blood.

*When both calcium and phosphorus are reduced* below normal concentration in the blood serum, calcification is retarded, but the structural abnormality of the bone differs from that of the two types described above. The condition constitutes a *third type of rickets* under Park's definition, but because of the differences in clinical and histological appearance it is sometimes called an *osteoporosis* instead.

Unless the mineral supply is very deficient, *any of the three types of rickets can be prevented by vitamin D, which acts to restore to normal the concentrations of calcium and phosphate ions in the blood.*

Whence does the vitamin "mobilize" the calcium or phosphate or both?

The chief answer seems to be by prevention of intestinal losses. Thus Shohl states unqualifiedly that the main action of vitamin D is to increase the absorption of calcium and phosphorus or to diminish their intestinal excretion; and additional evidence that vitamin D functions at least in part by increasing the intestinal absorption of calcium and phosphorus was presented at the 1940 meeting of the American Society of Biological Chemists by Margaret Cammack Smith and Harry Spector.

This, however, may not be quite the whole story of the action of vitamin D in the prevention of rickets.

Sometimes the calcium or phosphorus mobilized through the

blood-stream and thus made available to the developing ends of the bones seems to come from stores already in the tissues.

There are also some indications that a local factor, affecting, as Hess has expressed it, the "anchorage" of the available calcium and phosphate ions in the end of the growing bone, may play a part in particular cases.

And more recently Schneider and Steenbock have offered evidence of a specific effect of the vitamin upon phosphorus metabolism such as to favor the deposition of calcium phosphate as bone mineral in the cartilage of the developing bone ends, at the expense of the soft tissues, thereby retarding the general growth of the body.

Park (1940) writes: "No one knows how vitamin D acts."

While scientific explanation is probably still incomplete, the problem of prevention of rickets is now sufficiently solved that few new cases of severe rickets are seen, and the mild rickets nowadays occurring may in large part be regarded as incidental to the fluctuating fortunes with which the soft tissues and the developing bones compete for the phosphate which the blood stream brings.

The prevention of rickets by the exposure of the body to sunshine or ultraviolet irradiation undoubtedly comes about through the production of "animal vitamin D" in the skin. Notice, too, the efficiency of the "mechanism" which at the same time increases the circulation of blood through the skin, bringing precursor and taking active vitamin into the body.

### **The Promotion of Growth and Development**

When severe rickets was more common, its outward signs of bow-legs and knock-knees often seemed more noticeable in the more rapidly growing children. This would be natural in so far as general growth means increase of soft tissue; and the more active the soft-tissue growth the less the supply of phosphorus for the deposition of bone salt which is essential to the normal hardening of the growing bones. If the bones are prevented from adequate hardening and at the same time are made to bear an increasing body weight the bending of the bones and enlargement of the joints will be worse, the greater the weight put upon them. Under these conditions, then, "the greater the growth, the worse the rickets." But, with the discovery and general use of vitamin D, the situation has largely changed; and, indeed, as mentioned above,

there is now the suggestion that liberal dosage with vitamin D may so divert phosphorus to the developing bones as to check the growth of the soft tissues.

What chiefly needs emphasis at present is the importance of liberal nutritional intakes of calcium, of phosphorus, and of vitamin D.

When the intakes of calcium and phosphorus are abundant to the needs of the growth and development of both soft tissues and bone, liberality of vitamin D tends to promote growth; more especially linear growth, *i.e.*, increase in the length of the skeleton, perhaps especially of the legs.

The superior height and erectness of carriage which in some countries has hitherto been considered "a characteristic of the upper class" is due to long straight legs and is now being largely conferred upon the children of all economic conditions through the discovery of vitamin D and the widespread use of fish liver oils. (This is quite as important a service of chemistry to democracy as was Dr. Slosson's favorite illustration, that through the cheap synthesis of the dyestuff "every working-girl can now wear Royal Purple.")

Like many other of the constructive developments of the newer knowledge of nutrition, the favorable influence of liberal intakes of vitamin D upon growth, when intakes of calcium and phosphorus are also liberal, was first shown by experimentation with laboratory animals of short natural life cycle and then confirmed and extended by clinical experience.

In addition to its rôle in the prevention and cure of rickets, vitamin D was found (Sherman and Stiebeling, 1929, 1930) to promote growth and to facilitate the normal calcification in developing bone even when the mineral metabolism is well above the rickets level.

Together with other factors, vitamin D is of importance in the formation of normal teeth and protection against dental caries.

There is clinical evidence that children who have been protected from rickets by cod liver oil are subsequently less susceptible to respiratory disease than those who have had rickets, but it remains an open question in how far the stronger respiratory system is due to the vitamin D and in how far to the vitamin A which the cod liver oil furnished.

The well controlled clinical investigations of Jeans and his co-workers are now (1940) bringing ever-stronger evidence of the



promotion of lateral growth in children by liberal allowances of vitamin D *when added to diets of liberal calcium and phosphorus content.*

Highly significant is the repeated emphasis laid by Jeans and Stearns (1939) upon the fact that while vitamin D is in this respect perhaps even more helpful to physical development than heretofore realized, one's confidence in the vitamin must never be allowed to make one less carefully attentive to the importance of liberal calcium intake. Increased calcification means increased retention of calcium in the body, and the body must receive large amounts in order to retain large amounts.

The vitamin must never be expected to decrease the calcium requirement. Rather, it helps the body to make optimal use of a generous calcium intake. The intake of calcium must always be generous if optimal calcification and growth of bone are to be secured.

### **Storage of Vitamin D in the Body and Its Transfer from Mother to Young**

Both carefully controlled laboratory experiments with rats and clinical experience with mothers and infants have shown that the antirachitic vitamin can be stored in the body, and to a very important extent. The nutritional condition of the mother influences the storage of vitamin D in the body of the baby before its birth, and her bodily store of this vitamin (or lack of it) affects the antirachitic potency of her milk.

Vitamin D taken by a pregnant or nursing mother is, therefore, a nutritional asset both to herself and to the child.

Hess and coworkers encountered cases in which young rats which they had intended using as test animals proved "refractory to rickets," because of the highly antirachitic character of the mother's diet, and the fact that the litters were small. This, of course, is a converse demonstration of the clinical experience that severe rickets is more frequent among the children of the poor whose mothers are living on food of inferior mineral and vitamin content, often in dark tenements, and also have borne and suckled many children.

### **Measurement and Expression of Vitamin D Values**

Vitamin D values are generally measured either by means of the "line test" or by determination of calcium or total ash in a representative bone.

McCollum and his coworkers have found in their autopsies of rats which had been on deficient diets, that a rickets-producing diet of a sufficiently drastic character may result in a condition in which the cartilage and adjacent portions of the metaphyses of the long bones are entirely free from visible deposits of calcium phosphate. If, then, such rats are given an effective antirachitic treatment of any kind, there results within a few days a line of freshly laid down deposits of calcium phosphate which by proper staining can be made clear to histological examination. This was (and is) called the *line test*. Bills, Honeywell, *et al.* (1928, 1931) elaborated the technique and gave a very full description of it as used by them. It was also adopted by Steenbock and his coworkers for the routine testing connected with their control of the manufacture of irradiated foods and drugs under the Steenbock patents.

The line test has been adopted as the basis for the *United States Pharmacopeia* unit for vitamin D potency. Present procedure provides for the comparison of the degree of healing caused by the test material with that caused by a known quantity of a standard reference cod liver oil in a similar group of animals under identical conditions. In this way the effects of variations in the susceptibility to rickets of different groups of rats, due to environmental and other factors, are eliminated. The standard reference cod liver oil, which is distributed by the *Pharmacopeia* to laboratories where an assay for vitamin D is to be carried out, has been checked against the International vitamin D standard, adopted by the League of Nations Health Organization, so that one U.S.P. unit is equivalent to one International unit of vitamin D.

The international standard of vitamin D is a solution of irradiated ergosterol, made under defined conditions, and of a strength corresponding with 1 mg. of the original ergosterol activated by irradiation and dissolved in 10 cc. of olive oil. The unit of vitamin is 1 mg. of this solution; and the activity is such that 1 mg. given daily to rachitic rats for 8 successive days should produce a wide calcium "line."

It is well to realize that, in order to produce rickets in rats for the line-test method of assay, greatly unbalanced mineral intakes are employed, the usual rachitogenic diets containing four to five times as much calcium as phosphorus, which is a very much wider ratio than is likely to be encountered in human experience.

Stiebeling has employed a different procedure in which the basal

diet fed the experimental animals (rats) is adequate in its mineral content as well as in all organic factors other than vitamin D, and in which the vitamin D value of the material under investigation is judged by the relative amount necessary to allow a "standard" degree of calcification under fixed experimental conditions.

Young rats receiving the basal diet only (and no irradiation) serve as "negative controls"; those receiving the basal diet plus abundant vitamin D (or irradiation) serve as "positive controls"; others receive the basal diet plus graded allowances of the food or other material which is being tested for vitamin D value.

At the end of a four or five week experimental period the degree of calcification is found by determining the percentage of ash or of calcium in the freshly removed femur. Within certain limits, this is found to be dependent upon the relative amount of vitamin D supplied. The advantage in calcification shown by the test animals over the negative controls must be due to the material being tested, provided the experiments are properly conducted and in sufficient numbers to avoid vitiation of results through individual variation. It is suggested that the "end point" for comparison be one half as much improvement in calcification over negative controls as is produced by supplying an abundance of vitamin D (positive controls). The amount of the food needed to "reach this end point" (maintain this standard rate of calcification) may be taken as inversely proportional to its richness in vitamin D.

### The Human Requirement of Vitamin D

This section is essentially based upon the treatment of the same subject by Jeans and Stearns (1939). They consider that for many persons some vitamin D in addition to that ordinarily obtained by exposure to sunshine is necessary for optimal use of calcium and phosphorus in the body. This may be obtained by special irradiation of the skin, by use of such foods as contain vitamin D, by the taking of the vitamin in some more concentrated form, or in a combination of these ways.

Jeans and Stearns point out that we should recognize three phases of the vitamin D requirement: (1) that for normal growth and mineralization\* of the skeleton, including the teeth, of infants and children; (2) that for maintenance of these skeletal structures

\* Mineralization is a useful word to remind us that the calcification of the bone tissue involves a deposition of bone *mineral*. It is worth while to preserve the dis-

in sound condition throughout adult life; and (3) that to supply the special needs of pregnancy and lactation.

There is as yet no consensus of scientific opinion as to what constitutes a normal or optimal rate of mineralization of the developing skeleton. Jeans and Stearns state that this is most rapid in early life, and that among the estimates of desirable calcium retention "the higher figures are thought to be a safer guide."

*During infancy* the vitamin D requirement depends to some extent upon the rate of growth. The more rapidly the infant grows the more important it is to safeguard him against rickets: also the liberal allowance of vitamin D, given as such a safeguard, itself tends to increase the rate of growth (Jeans and Stearns, 1939, p. 486). Infants whose daily food includes a quart of milk containing 135 units of vitamin D seem to be fully protected from rickets; but additional vitamin D up to a total of 300 to 400 units seems to support a rate of growth and development "nearer the top of the normal range." See chart quoted by Jeans and Stearns (1939, p. 488) from Slyker, Hamil, Poole, Cooley, and Macy: *Proc. Soc. Exptl. Biol. Med.* **37**, 499.

It also appears, from the observations of Jeans and Stearns and of Macy and coworkers, that increasing allowances up to an undetermined level between 135 and 300 units of vitamin D per day tends toward a more uniform ability among infants to assimilate calcium from the food. This appears to be true even of infants fed human milk (Jeans and Stearns, 1939, p. 493).

*During childhood* there is need of continued emphasis upon liberal intakes of calcium and of vitamin D if optimal development of bones and teeth is to be maintained. As yet (Jeans and Stearns, 1939) the needs of children in this respect do not seem to have been as fully realized as the needs of infants, probably because it is less easy to make the child's need obvious. Beyond emphasizing the view that there should be no let-down of calcium or vitamin D intake as infancy develops into childhood, little can yet be said as to the child's quantitative need.

Jeans and Stearns have studied fifty children aged 1 to 12 years with varying intakes of milk and with and without added vitamin D in the form of codliver oil to the extent of 300 to 400 added units

tion (sometimes neglected in nutritional writings) between a mineral and a mineral element. The iron of hemoglobin, for instance, is a mineral element but not a mineral.

of vitamin D per day. They found that children varied in their need. If a child utilized calcium efficiently without the added vitamin D, the addition of this to the diet had little effect; while if the original utilization was poor, the ingestion of additional vitamin D increased the retention of calcium.

*Whether adults need vitamin D and if so to what extent* are questions upon which there is little evidence beyond the fact, so carefully and repeatedly emphasized by Jeans and Stearns, that vitamin D does not lower the minimum requirement for ingested calcium.

*In pregnancy and lactation* it is undoubtedly wise to be liberal with the vitamin D allowance; but perhaps to be scientifically candid we should say that the proposed "standards" for pregnant and nursing women represent (1940) liberality of attitude rather than precise quantitative knowledge. Much more certain is the fact that the need for calcium is markedly increased in the latter part of pregnancy and during lactation. (See Jeans and Stearns (1939, page 508) and other writings there cited.)

### Sources of Vitamin D

The antirachitic values of fish liver oils, of irradiated ergosterol, and of ultraviolet irradiation of the body, are so great that they have absorbed attention to perhaps a greater extent than is desirable. The presence of important amounts of vitamin D in egg yolk, whole milk, and butterfat has, however, been established. That in some cases the evidence of its presence has failed to appear is probably due in large part to the use in many experiments of so drastic a rickets-producing diet that symptoms of rickets developed in the experimental animals notwithstanding the presence of amounts of vitamin D (in the food fed for test) which would have been measurable under more nearly normal conditions. Mellanby, working with puppies and using basal diets not so drastically deficient as are the rickets-producing diets employed by most of those who work with rats, had no difficulty in showing the vitamin D value of green vegetables and of butterfat. Shipley, Kinney, and McCollum (1924) showed the presence of vitamin D in ether extracts of green leaves. Also in the work of Sherman and Stiebeling which demonstrated a considerable vitamin D value in milk, the diets were so arranged and controlled that the effect of the milk in promoting deposition of calcium phosphate in the growing bone must have been due to the vitamin rather than the calcium

or phosphorus factor of the food, since the amounts and ratios of calcium and phosphorus in the intake were strictly controlled.

As was seen to be true of vitamin A, so also with vitamin D there seems to be survival value for the species in the capacities that have been developed by the maternal organism to collect the vitamin and pass it on to its young through the fat of the egg yolk or of the milk as the case may be. The concentration is higher in the egg fat, but the total amount of vitamin D obtained through the milk fat is considerable because of the consumption of the latter not only in the form of milk itself but also of cheese, butter, cream, and ice cream. Vitamin D is sufficiently stable, except under irradiation, so that no serious loss need be feared in the making or storing of such foods as these, or in ordinary cooking operations. Direct experimentation has shown its stability at autoclave temperatures.

*Vitamin D milk* is the term commonly used for market milk of enhanced vitamin D value.

Investigators differ as to the practicability of materially increasing the vitamin D value of milk by the irradiation of the cow.

Three methods of enrichment of milk in vitamin D are in practical use: (1) the addition of a concentrate of natural vitamin D; (2) feeding irradiated material to the milch cow; (3) irradiation of the milk itself.

The choice between these three methods depends partly on economic factors, and partly upon considerations having to do with the differences in kinds and relative proportions of the vitamin D or vitamins D introduced by the respective methods.

Whatever may be the ultimate consensus of opinion as to the relative merits of these different forms of enrichment, it is already established that vitamin D milk is a highly effective means of administering vitamin D.

With milk of such greatly enhanced vitamin D value now generally available, and with the wide general distribution of codliver oil, of the more potent liver oil of the halibut, and of the still more potent *percomorph liver oil*, it is proper enough to place chief dependence upon these highly potent and now well standardized sources, in comparison with which the natural vitamin D values of foods are often treated as if insignificant, — or even stated to be so. But the natural vitamin D values of foods are scientifically significant to our understanding of the processes of nature and how

children got along even as well as they did before vitamin D was discovered.

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## CHAPTER XXIV

### OTHER FAT-SOLUBLE VITAMINS

#### Vitamin E (Tocopherols)

ALMOST simultaneously with the full establishment of vitamin D as a fat-soluble essential distinct from vitamin A, different investigators prominent among whom were Evans and his coworkers at the University of California and Mattill, working first at the University of Rochester and later at Iowa University, found evidence that for normal reproduction a further fat-soluble factor was nutritionally essential. This came to be called vitamin E, then *tocopherol*, and more recently *alpha-* and *beta-tocopherols*.

The three known natural tocopherols are so closely related that custom continues to use the collective-singular term, vitamin E.

Evans' structural formula for alpha-tocopherol is shown in Fig. 42.

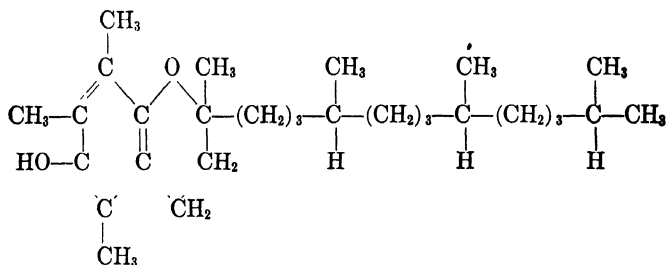


FIG. 42. — Alpha-tocopherol.

Vitamin E is rather widely distributed in nature where it is largely associated with anti-oxidants (Bradway and Mattill, 1934; Olcott and Mattill, 1931, 1934).

Chemically, this association with anti-oxidants in natural occurrence correlates with the fact that vitamin E, while relatively stable in several respects, is rather easily destroyed by oxidation.

The oxidative destruction of vitamin E is catalyzed by iron salts. Hence a food mixture for experimental purposes can be made deficient in vitamin E by moistening with a solution of iron salt and then heating to dryness.

When rats are fed diets otherwise adequate to their needs but lacking vitamin E, the males become permanently infertile through an irreversible degeneration of the germinal epithelium; females fail in reproduction, the embryos being resorbed, but the female reproductive system does not suffer permanent injury.

Evans (1939) emphasizes the occurrence of degeneration of the cross-striated or voluntary musculature in vitamin-E deficiency, but considers it not yet clear whether this is a primary effect upon the musculature or comes about secondarily after primary injury to the nerves.

In turn, Pappenheimer's studies of vitamin-E deficient chicks indicate that here the injury to the nervous system is the result of an impairment of the blood vessels supplying the brain.

Puppies, rabbits, guineapigs, and rats have developed muscular dystrophy when kept for long periods on vitamin-E deficient diets, and in some cases this has been cured by giving synthetic alpha-tocopherol.

Vitamin E occurs abundantly in wheat germ oil, but may not be its only influential constituent as has been assumed by some experimenters.

Because of its wide distribution in foods and the improbability of its being a limiting factor in human nutrition, it seems probably unnecessary and possibly misleading to lay emphasis upon vitamin E in practical considerations of food values. Reports of cases in which vitamin E has seemed to be helpful in the treatment of certain muscular and nervous diseases are included among the references at the end of this chapter; but it should be noted carefully that the Council on Pharmacy and Chemistry of the American Medical Association, in an equally recent statement, has warned against regarding the value of vitamin E for man as established, and has emphasized the need of more fully controlled investigation.

For a fuller account of vitamin E, the paper of Mattill (1939) may conveniently be consulted; and for later discussions of its molecular structure see Smith (1940).

### "Vitamin F"

For a time the term *vitamin F* was applied to the nutritionally essential fatty acid (or acids), and may still be met commercially in that connection. Scientists, however, decided against this ter-

minology, and it is no longer used in scientific literature nor recognized as a therapeutic name by the medical profession.

### Vitamins K<sub>1</sub> and K<sub>2</sub>

As early as 1929 it had been observed by the Danish investigator Dam that chickens raised on certain artificial diets were subject to subcutaneous and intramuscular hemorrhages; and further study of such a hemorrhagic chick showed an abnormally long time required for the clotting of the blood. Others confirmed these observations; and Dam (1935) extended his work to the finding of experimental evidence that the hemorrhagic tendency and abnormally prolonged clotting time were attributable to shortage of a fat-soluble "vitamin K" (from the Scandinavian and German term "Koagulations-Vitamin").

The work of several investigators showed that such a factor is widely distributed in green leaves.

One form of antihemorrhagic vitamin, obtained from alfalfa leaf meal and designated as vitamin K<sub>1</sub> or as *phyllochinon* (*phylloquinone*), has been identified chemically as 2-methyl-3-phytyl-1,4-naphthoquinone. (Its structural formula is shown in Fig. 43.)

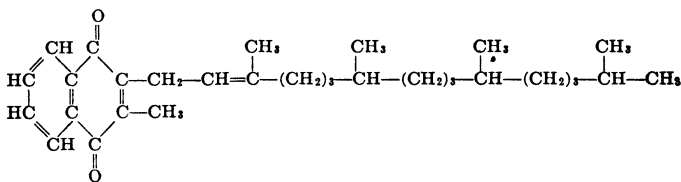


FIG. 43. — Vitamin K<sub>1</sub>  
2-methyl-3-phytyl-1,4-naphthoquinone.

A second form of antihemorrhagic vitamin was isolated by Doisy and coworkers from putrefied sardine meal. This vitamin K<sub>2</sub> is believed by them (Binkley, McKee, Thayer, and Doisy, 1940) to have a molecular structure like that of vitamin K<sub>1</sub>, except that in place of the phytyl group there is a longer, more highly unsaturated side chain.

In addition to these forms of vitamin K known to occur naturally, many synthetic products show antihemorrhagic properties in greater or lesser degree. Some of these are, — like vitamin K<sub>1</sub> and probably also vitamin K<sub>2</sub>, — 3-substituted-2-methyl-1,4-naphthoquinones, while others are less closely related in structure.

References to numerous investigations of this subject are to be found at the end of the chapter.

Bile salts appear to be highly important if not absolutely necessary to the absorption of (natural) vitamin K. For this reason, patients with diseases of the biliary tract in which bile flow to the intestine is impaired are apt to develop a condition of low blood-clotting ability which is really a K-avitaminosis, notwithstanding the fact that their diet may have contained normally adequate amounts of antihemorrhagic factor. Numerous clinical tests show that the hemorrhagic tendency in such conditions may be controlled either by the oral administration of bile salts with vitamin K concentrates or by the injection of antihemorrhagic vitamin.

The low clotting-power of the blood in vitamin K deficiency has been traced to an abnormally low content of *prothrombin* (a normal protein constituent of the blood which, with calcium ion and the cephalin-containing thromboplastic factor, gives rise to blood clot). Hypoprothrombinemia has been found clinically in various conditions of impaired digestive function and treated effectively in most instances as a K-avitaminosis. Since diminished clotting power due to low prothrombin content appears to be usual in the blood of newborn infants, some clinicians recommend administration of vitamin K either to the mother before delivery or to the infant at birth.

For fuller accounts, see the excellent reviews of Dam (1940) and of Brinkhous (1940).

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## CHAPTER XXV

# THE NUTRITIONAL CHEMISTRY OF REPRODUCTION AND LACTATION

### Endocrinology Recognized as an Autonomous Science

AMONG the materials which the nutritional process provides for the body are those from which the specific substances of the internal secretions are formed. Endocrinology, however, has developed to such an extent as now to be recognized as a distinct field of study. While the actual bodily processes of the internal secretion of active substances must obviously have a nutritional basis, the science of endocrinology is recognized as autonomous and the subject-matter of this chapter will not include the study of the endocrines concerned in reproduction and lactation, but only (1) the quantitatively increased demand for nutriment which reproduction and lactation involve, and (2) the problem of the extent to which the meeting of the nutritional need of the offspring is automatic under physiological regulation.

### Nutritional Demands of Pregnancy

We do not know that the food of the expectant mother need furnish any different thing from the nutrients required for growth and maintenance, but it is clear that the function of gestation increases the nutritional need, and undoubtedly in somewhat different proportions for different nutrients.

Maternity hospital records reveal that women classified as ill-nourished during pregnancy show greatly increased proportions of still-births, of premature births, and of deaths of babies within ten days after birth.

According to Mendenhall: "During pregnancy, proper prenatal hygiene and feeding is essential to the life and normal development of the unborn child. To count on the fact that the mother is a factor of safety in the nutrition of the young, and to nourish the child either at the expense of the pregnant or nursing mother, is an

unnecessary sacrifice of the woman and may at any time prove disastrous to the child." And also, "there is every reason to believe that ability to produce breast milk of a superior quality is to some extent dependent on the storage of material from the mother's food during the prenatal period, as well as on the supply of nutrients from the food she receives during lactation.")

The increase in the energy requirement becomes measurable after about four months of pregnancy and then rises steadily until at term it is about 25 per cent greater than for the same woman during reproductive rest. (See also Chapters IX and X.)

Hunscher *et al.* (1935) found that with generous amounts of proteins in the diet, the maternal organism tended to store nitrogen greatly in excess of the requirements of the fetus and its adnexa; and that this maternal reserve acted to offset the strongly negative nitrogen balances of the subsequent (in the case which they studied, unusually heavy) lactation period. On the other hand, Coons has found in pregnant women who chose diets considerably lower than usual in protein content (about 11 grams of nitrogen per day) that the nitrogen retentions were not in all cases as high as the amount presumably transferred to the fetus; and there were indications that low storage of nitrogen during pregnancy might adversely affect lactation.

The amounts of material actually transferred into the body of the fetus have a bearing upon the present problem. As an average of rather widely varying reports, it appears that something like 60 grams of nitrogen, 20 grams of calcium, and 15 grams of phosphorus are contained in the body of the normal infant at birth. Obviously, if the mother's positive balances during pregnancy should fall short of the amounts with which the baby is born the maternal organism must have been depleted. This is, perhaps, more often true of calcium than of other elements thus far studied. Thus Coons and Blunt (1930) found that a group of Chicago women, each choosing her own diet, stored during pregnancy less calcium than the fetus was estimated to contain.

It does not follow, however, that the materials contained in the body of the baby at birth represent the whole of the increased need of pregnancy over simple maintenance. Macy and Hunscher (1934) have computed that under favorable nutritional conditions, the positive balances during pregnancy amount to about 500 grams of nitrogen, about 65 grams of phosphorus, and about 29 grams of

calcium (or 700–800 per cent more of nitrogen, 300–400 per cent more of phosphorus, and about 25–50 per cent more of calcium than is contained in the actual body of the baby). To an extent not yet clearly defined, these surpluses appear to represent the laying up by the mother during pregnancy of nutritional reserves against the heavy demands of lactation. Macy and coworkers (Hummel *et al.*, 1936) show by prolonged balance studies the practicability of providing through the food for such liberal storage of calcium, magnesium, and phosphorus (as well as of nitrogen).

In the chapter on iron requirement, the increased need during pregnancy has been explained in general terms. An attempt to treat it in a more quantitative way would probably be premature, as the estimates of the amounts contained in the normal fetus and retained during a normal pregnancy are as yet too variable to afford a sound basis for interpretation.

In regions in which the environment provides no liberal margin of iodide intake (in general, the goitrous regions), iodine deficiency may be induced by the extra demands of pregnancy and lactation. Medical advice should be sought in safeguarding against this danger.

Vitamin requirements are undoubtedly increased in pregnancy, but we have no ground for assuming that the increase is the same in the case of each vitamin. As has been noted in earlier chapters, these substances are not a natural group: they differ so much both in chemical nature and in nutritional function that the only scientifically sound approach is to study each separately upon its own merits, making no assumption whatever that the findings for any one vitamin will be true for any other.

Clinical observations upon expectant mothers are now generally interpreted to indicate an increased need of thiamin in pregnancy.

Of riboflavin, the allowance should probably be at least equally generous, though the evidence is of a different kind. Large numbers of long-continued experiments with laboratory animals have shown (Chapter XIX) that increased intakes of riboflavin by the mother continue to confer increasing benefit upon the offspring up to unexpectedly high levels. Further studies of this phenomenon are in progress at the time of writing (1940).

The findings of Batchelder (Chapter XXII), now being extended by Campbell and coworkers, show that, while the mechanism of its action must be very different, vitamin A also gives increasingly

good results for increasingly liberal intakes by the mother up to levels much above that of minimal adequacy.

Again, it would be unscientific to generalize from riboflavin and vitamin A to other vitamins; for riboflavin and vitamin A were chosen first for long-term study, not at random but because of special indications arising from full-life, successive generation experiments in which the units of experimental variation were articles of food (Chapter XXVIII).

### **Division of the Penalty of Malnutrition between the Mother and the Offspring**

To a large extent, the organism of the mother serves as a "factor of safety" in the nutrition of the young both before birth and during lactation. Occasionally, however, this important principle, — that Nature is "more careful" of the perpetuation of the race than of the conservation of the adult whose parental function has been performed, — may be overemphasized. Thus it is an exaggeration to say that the unborn offspring "is a *perfect* parasite upon the mother." It is in a sense a "parasite" but not a "perfect" one in the sense of taking just as much from the mother whether she has it to spare or not.

The fetus may draw heavily upon the mother, taking material beyond that which the mother can well spare if not well nourished; but when the nutritional intake is seriously deficient to the total demand of pregnancy it must be expected that both the mother and the young will be unfavorably affected.

This is illustrated in the classic experiment upon young milch cattle at the University of Wisconsin. Four groups of young females were placed on four rations derived respectively from the wheat plant, the oat plant, the corn (maize) plant, and a mixture of the three. By mixing proper proportions of the leaves and stalk of the plant with its seed (and when necessary supplementing with some of a protein-concentrate of the same seed) all four rations were given the same energy and protein content. Growth was completed on all four of these rations; but the reproduction and lactation records were very different. To simplify the summary by making use of present knowledge, it may be said that the succulent stalk and green leaves of the maize so supplemented the seed as to make a diet which supported reproduction and lactation well, while the woody stems and scanty leaves of the wheat and



oats did not fully meet these nutritional needs. The difference was not in the grains, for wheat or oat grain fed with corn stover (tops and leaves of the maize plant) or with alfalfa hay gave good results (Hart *et al.*, 1917).

Later experiments showed that the wheat plant ration could be made adequate for reproduction and lactation by supplementing it with bone meal and cod liver oil. Hence the explanation would seem to be in the enrichment of the diet in one or more of the following chemical factors: calcium, phosphorus, vitamins A and D, riboflavin, and possibly in small part the additional protein of the bone meal.

Of these factors, a large amount of evidence from other feeding experiments shows calcium and vitamin A to be highly important here as in other phases of nutrition. Phosphorus also is important, but much less likely to be a limiting factor in a case like this in which liberal amounts of whole grain were eaten. Whether vitamin D had any such far-reaching importance as vitamin A in these experiments cannot be stated. It is possible that some of the amino acids from the extra protein furnished in the bone meal may have improved the ration for lactation, since lactation seems to increase this phase of nutritional need. And it is altogether probable that reproduction and lactation were aided by such extra riboflavin as the bone meal furnished.

This chemical explanation is also consistent with the fact observed in the Wisconsin experiments that similar improvements were obtainable by the feeding of the bone meal and cod liver oil on the one hand or of alfalfa hay on the other. In terms of human nutrition, the corresponding chemical enrichments would best be effected by the use of increased amounts of milk and green vegetables.

What the psychologists tell us is doubtless true, — that we have a keener awareness for disasters than for benefits. Having illustrated, by means of a striking experiment in malnutrition, the principle of community of nutritional interest in mother and young, we should also say with at least equal emphasis that the principle applies to benefits as well as to disasters.

In experiments with laboratory animals success in the launching of the succeeding generation is often found to be an exceedingly valuable indication of nutritional well-being and resulting vitality. A diet which results in a superior record of reproductive success

when fed to a properly selected laboratory animal may be expected to contribute to the attainment of a superior nutritional condition in the human with resulting higher efficiency in whatever line of human endeavor. The use of the reproduction record as one item in a connected series of criteria of improvement in nutritional condition is well illustrated in the work of Campbell (1928, 1931).

Further research in the Columbia laboratories is showing unexpectedly constructive possibilities in this line of nutritional research.

Because of greater individual variability in reproductive success than in most other aspects of the life history, and because success in the reproductive segment of the life cycle is influenced not only by the nutritional intakes at the time but also by the whole nutritional history from before birth, the accumulation of knowledge in this field by observations upon human experience would be a discouragingly slow process and open to misinterpretations or mistakes of emphasis, if it were obliged to stand alone. Hence the very great value of controlled experimentation with laboratory animals, small enough to be used in large numbers so that findings may be safe from distortion by individual variations, and of sufficiently short natural life cycle so that experiments may be continued under accurate control for the entire lives of two or more generations.

In such experiments it has often been found that the limiting nutritional factors of reproduction and lactation are not specific but are the same which may also limit growth, and that reproduction and lactation make especially large demands for these nutritional factors. In many cases it will be found that a dietary or ration of such nutritional character as to show only slightly subnormal results in growth will yield more markedly subnormal results in reproduction and lactation. (Some of the far-reaching human implications of such research are briefly considered in Chapter XXX.)

### **General Interrelations of Nutrition and Lactation**

As explained at the beginning of this chapter, there is an important and rapidly developing field of knowledge which is nutritional in the broad sense of this word, but which, being also within the province of endocrinology, we leave to the endocrinologist in our present study of nutrition. The paper by Nakahara

and coworkers (1938) on vitamin L, the "lactation vitamin" or lactagogue, included in the list at the end of the chapter belongs to this borderline field; as does also that of Bates and Riddle (1935), while many others from the highly fruitful work of Riddle and his associates must here be omitted from individual mention.

### Variations in Quantity and Composition of Milk

In cases in which a young mammal is entirely dependent upon its mother's milk, it may show nutritional deficiency if the milk is subnormal either qualitatively or quantitatively.

In cases in which cows have been fed rations very deficient in some vitamin, and their milk has then been fed to rats or guinea-pigs to test its vitamin value, even a large decrease in the quantity of milk produced would often be ignored as having no bearing upon the immediate experiment; for there would still be enough milk for the feeding of the small experimental animal, so that only the quality of the milk as regards the vitamin under investigation would be brought into discussion. Such experiments have served the useful purpose of showing that, in the main, the ultimate dependence of the vitamin value of the milk is chiefly upon that of the food.

But in general experience, feeding has more effect upon the quantity than upon the quality of the milk produced. If there are cows so badly fed as in the experiments with vitamin-deficient diets here referred to, they will produce so little milk that its quality can have little effect upon the average quality of the milk sent to market. And the feeding of such a vitamin-deficient diet would not be likely to become prevalent in modern dairy farming because it would be too unprofitable. In actual practice, human milk is apt to be more variable than cow's milk in this respect, because the human mother is apt to be less systematic in feeding herself than the modern dairy farmer is in feeding his cows.

The milk of different species contains essentially the same substances but in somewhat different concentrations. The treatise entitled *Fundamentals of Dairy Science* by the Associates of Rogers summarizes the results of a great deal of research relating primarily to the production and composition of cow's milk, but having very important bearings upon human lactation also.

In general, milk contains a rather variable proportion of fat dispersed in the form of globules floating in a serum or plasma which

contains proteins, lactose, and salts in much less variable proportions.

Regarding individual or temporary variations in lactose or salt content, Van der Laan found that the osmotic pressure of milk is essentially the same as that of the blood from which it is secreted, and is, therefore, nearly constant. In milk this osmotic pressure is almost entirely due to the lactose and the soluble salts; hence, a shift in the concentration of either lactose or soluble salt in the milk is accompanied by a reciprocal shift in the other. Usually, however, such variations are a rather temporary result of ill-defined physiological variation.

The calcium and phosphorus contents of milk are more stable. In Chapter XIV we saw that the averages of over 200 quantitative determinations of each of these elements show very small probable errors, and that the coefficient of variation is in each case only 7.

Apparently, the concentrations of mineral constituents are somewhat different in colostrum than in milk. Garrett and Overman (1940) report that the colostrum of the first few hours is richer than milk in calcium, magnesium, sodium, phosphorus, and chlorine, while poorer than milk in potassium; but that all these elements quickly reach their normal milk concentrations as the other properties of colostrum change to those of milk.

Milk has, as we have seen in Chapter XXII, a fairly high vitamin A value, due (both in the case of the human mother and of the cow) in part to vitamin A itself and in part to carotene. Colostrum has a vitamin A value several times as great as the milk secreted later, and is relatively very much richer in carotene than milk. The high vitamin A value of milk and particularly of colostrum is of great importance for the infant, whose body stores of vitamin A at birth are, even under favorable conditions, probably low in comparison with those of well-nourished adults.

The vitamin A value of milk depends ultimately upon the dietary intake of this factor and its precursors, but the storage of vitamin A in the body tends to equalize the amounts secreted from day to day. However, cow's milk produced during the winter by stall-fed animals with only a limited supply of carotene-containing foods may show a vitamin A value distinctly lower than that produced during the summer months when the cows are pastured and consequently have access to abundant amounts of carotene in the green grasses. McCosh *et al.* (1934) found that

the addition of 15 grams of cod liver oil daily to an already excellent (and probably far better than average) human maternal dietary failed to improve the vitamin A value of the milk produced; but other experiments, with dietaries of lower vitamin value as a starting-point have indicated that a generous allowance of vitamin A in the diet of the mother increases the concentration of this factor in her milk.

It is a fact, long familiar to dairymen, that in drawing the milk which can be obtained at any given time, that which is yielded first is relatively poor in fat and that yielded last is of much higher fat content, while the aqueous phase of the milk is of very nearly constant composition throughout. It is now recognized that this fact can be utilized (when desired) in the breast-feeding of infants. If the infant is allowed to suckle the first portions of the milk available in the breast but not the last portions, he gets a diet of diminished fat content, while in the reverse case the fat content of his diet is increased. In either of these cases his vitamin A intake is correspondingly decreased or increased. But a change to a different wet-nurse whose milk is regularly higher in fat would not necessarily mean an increase in the vitamin A intake.

This is but one of many ways in which facts which have been established through research upon lactation in dairy animals may now be utilized to render breast-feeding more scientific.

Because of the bacterial synthesis of thiamin in the rumen, the cow (unlike the human mother) need not depend entirely upon her diet to supply thiamin for secretion into the milk, whereas in the human mother, a dietary lack of this factor is soon reflected by secretion into the milk of subnormal amounts of thiamin, and may result in the appearance of distinct symptoms of deficiency in the nursing infant. However, it has been shown at the University of Wisconsin for cow's milk, and by Macy and Donelson for human milk, that, starting with a normally good maternal diet, the addition of liberal amounts of thiamin fails to increase significantly further the thiamin value of the milk produced.

The probability that cows, or bacteria in the cows' digestive tracts, can synthesize vitamin C was suggested by the finding at the University of Minnesota that heifers, fed from birth on a diet which produced scurvy in guineapigs, secreted vitamin C into their milk. It is not clear to what extent dietary changes, such as those to which cows are normally subjected, alter the vitamin C

value of the milk produced. Human milk produced under favorable dietary conditions has been found to be several times as rich in vitamin C as ordinary cow's milk, but when the maternal diet is inadequate, the value may drop to subnormal levels.

The amounts of riboflavin in human milk and in cow's milk vary somewhat in response to the dietary intake, although the increases which have been induced (in both species) by feeding of materials especially rich in this factor have been only of the order of 50 to 75 per cent above the level observed under usually favorable conditions of feeding.

In contrast to the vitamin A and other nutrients of milk, the vitamin D value is more responsive to the ingestion of large amounts of the vitamin in addition to a usually good diet. However, the transfer of antirachitic vitamin is quantitatively not very efficient (only about 4 per cent finds its way through the cow into the milk) so that it may be more practical to add the additional vitamin D directly to the milk itself.

*Effects on milk production of deficiencies in the food supply.* Meigs' excellent discussion of this topic in the reference book already cited\* should be read in full by those who are interested in this aspect of lactation.

### Nutritional Support of Lactation in the Human Mother

Direct experimentation upon milk production has usually shown that not more than two thirds of the extra calories fed are recovered in the milk, or conversely, that the extra food requirement (in calories) is at least 50 per cent greater than the number of calories in the milk produced. In the work of Shukers, Macy, *et al.* (1931) it was found that: "Lactation increases the food demands (of the human mother) approximately 60 per cent over and above those of pregnancy." The actual voluntary food intakes found in two successfully lactating women were 4600 and 4800 Calories per day, respectively. (See also Chapters IX and X.)

In cases in which the physician believes that liberal feeding of the mother may help in establishing the activity or promoting the productivity of her mammary glands, it is doubtless wise (at least until knowledge in this field becomes more precise) to make the dietary liberal in respect to all nutritional factors; for the consensus of competent opinion is clearly in favor of the nursing of

\* Associates of Rogers (1935).

the baby by the mother, in all reasonably normal cases. And physicians are increasingly successful in stimulating human milk secretion.

Yet it may also be said that something in human evolution or in the conditions of present-day civilization seems to have worked at the cost of the natural function of human lactation, so that even with the best of nutrition the mother may not secrete as much milk as the baby needs. When the physician is thoroughly convinced that this is true of the individual case, the fact may be accepted without any undue feeling of defeat.

Liberal feeding for the support of lactation should begin early; for it can safely be inferred from the results of animal experimentation that the ability to sustain a good rate of milk production depends largely upon the nutritional condition induced and maintained by good feeding from the beginning of the lactation period (as well as previously during pregnancy). If the mother postpones her increase of food intake, or the provision of liberal supplies of mineral elements and vitamins in her food, until her milk production has begun to decline, the results are apt to be less good both for herself and for the baby.

It is also a safe inference from well-controlled experience with other species, that food intakes more liberal than the usually accepted standards for support of lactation, and the application of the newer knowledge of nutrition in the choice of food, may be very significant factors in inducing and sustaining a superior level of accomplishment in lactation.

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## CHAPTER XXVI

### SOME CHEMICAL ASPECTS OF GROWTH AND DEVELOPMENT

#### "Nature and Nurture"

IN THE first decade of our century, Waters used the words which have been so frequently quoted that they may be taken as expressing the point of view which prevailed for a generation: "The upper limit of the size of an animal is determined by heredity. The stature to which an animal may actually attain, within this definitely fixed limit, is directly related to the way in which it is nourished during its growing period."

Subsequent scientific evidence has somewhat modified the view that heredity predetermines the limit of an individual's or a family's development in quite such a "definitely fixed" manner. Like many other useful generalizations in science, Waters' formulation above quoted comes to require modification in order to be consistent with new scientific knowledge in its field.

The increased growth which Mendel and Hubbell (1935) found to progress through many generations of their animal colony could not have been predicted from the heredity of these animals, and that it was a phase of a real improvement in these families is shown by the fact that these same animals showed also an enhancement of adult vitality or stamina as reflected in the success of their reproduction and rearing of young. Similarly the improvement of an already adequate diet by Sherman and Campbell (1924, 1930, 1935) likewise proved favorable both to rate of growth, and to adult vitality, and in this case it was also shown to increase the length of adult life.

Todd (1935) wrote: "The adult physical pattern is the outcome of growth along lines determined by heredity but enhanced, dwarfed, warped or mutilated in its expression by the influence of environment in the adventures of life."

Speaking with extreme reserve and in the most conservative words as President of the Royal Society, Hopkins pointed out that

"nurture can assist nature" to a larger extent than has been supposed by the scientific thought of the past two generations.

### **Effects of the Levels of Intake of Different Nutritional Factors upon Growth and Development**

In whatever terms one chooses to state the change in our concepts referred to in the preceding section, the outstanding significance of the change is an awakening to the fact that the life history depends more upon the choice and use of food than hitherto believed.

Nutrition need not be merely a matter of supplying the pre-determined needs of an inherited mechanism. It can be more constructive than that. Thus it is something which in the light of our newest knowledge is seen to bear more responsibility than previous generations supposed.

There is need to discriminate among the different factors in nutrition: of some factors "enough is as good as a feast," while of other factors generous surpluses contribute to a better outcome. Also, of course, shortages of different factors produce different results. As some aspects are developed in other chapters, what follows here is largely in summary form, while a few points, not discussed in other chapters, are developed more fully.

### *Energy*

When a diet of such character as would ordinarily meet all requirements is fed to a growing animal but in amounts too small to meet the growth requirement, it is plain that such restriction may result in a deficiency of one, several, or all of the essential factors. If the diet is so selected as to be relatively rich in all the needed proteins, mineral elements, and vitamins, then restriction of the amount of food will result primarily (and perhaps solely) in an energy deficit. Waters described experiments which appear to have been of this character. He reported numerous cases of young cattle kept on restricted amounts of food of suitable kinds, the restriction being such as materially to retard the increase in weight as compared with that of a full-fed animal of the same age, or even to hold the young animal at stationary weight at an age when it should have been growing rapidly. In such cases of insufficiency of the total food (energy) intake the skeleton continues to grow, in

height at least, while adipose tissue steadily disappears, and the muscles become more or less depleted. In a young animal subjected to this type of under-nourishment the skeleton grows in height to a much greater extent than in width. Along with the narrower skeleton the underfeeding resulted in muscles of smaller diameter, absence of subcutaneous fat, and a general appearance of emaciation. Young animals thus held at constant weight when they should be growing are in reality undergoing starvation.

These experiments showed also that, within limits not yet at all well defined, retarded growth means retarded development of the organism. Thus an animal at twelve months of age and weighing on account of sparse nourishment only 400 pounds when it should under natural nourishment have weighed 800 pounds, does not have its tissues as fully developed and matured as they would have been had the nourishment been normal. For example, the flesh of steers 14 to 16 months old that had been sparsely fed throughout their lives presented the general characteristics of veal; whereas at this age the flesh of a highly nourished animal possessed the characteristic color, texture, and flavor of beef. Professor Eckles had shown that dairy heifer calves, when fed heavily, reach sexual maturity at from eight to ten months of age; whereas similarly bred individuals that were sparsely fed did not reach the stage of puberty under from 16 to 19 months of age.

Somewhat similar experiments were performed upon dogs by Aron. Here also, when the food was suitable in character but too limited in amount to support normal growth, the young animals grew in length and height but became thinner. Because of the "growth impulse" such an underfed young animal burns his reserve of body material to cover the deficit in the energy intake. Such a condition, continued indefinitely, results after a time in cessation of all growth and finally in death from starvation. A dog, which by underfeeding had been kept for a year at the weight which he had when 5 weeks old, had become long, tall, and very thin; when he was then fed liberally, he immediately gained in weight and circumference but appeared to have lost the capacity for further growth in length and height. If, however, the period of underfeeding be not too prolonged, the animal on subsequently receiving ample food may regain normal proportions and grow to full normal size.

Since stationary weight in the young animal which is attempting to grow with an insufficient energy supply does not mean cessation of all growth, it follows that the body of such an animal gradually changes in composition, the percentage of fat and per-

haps protein becoming less while the percentages of water and ash increase. If, however, the diet is rich in fat, as in experiments upon mice reported by Mendel and Judson, a simple diminution of the amount of food, to a point where gain in weight ceases, may not result in any such general replacement of fat by water, perhaps because in such a case the stunting may be due to insufficiency of some of the other factors rather than to an energy deficit.

The work of Winters, Smith, and Mendel, of Smith and Swanson, and Smith's review (1931) bring out differences of effects in stunting resulting from low-calorie, low-lysine, or low-mineral intakes, respectively.

The extended and interesting experiments of Maynard, McCay, and coworkers at Cornell were also planned from the point of view of restricting growth through a shortage of energy only. In these, however, the diet was so extraordinarily rich in protein and vitamins and the periods of retarded growth were so long that considerations not ordinarily important in nutrition were here made prominently influential. At the time of writing (1940) these experiments are still in progress, and until they have received the final interpretation of the investigators concerned, further comment from the viewpoint of this chapter would be premature.

### *Protein*

As explained in earlier chapters, it was shown by Osborne and Mendel that, with a diet adequate in all other respects, any one of a number of purified proteins such as casein, lactalbumin, or edestin may, if used in sufficient amounts, serve as the sole protein both for maintenance and for growth, while gliadin as sole protein food sufficed for maintenance but not for growth, and zein as sole protein did not suffice even for maintenance. Gliadin contains adequate tryptophane but only about 1 per cent of lysine; addition of more lysine to the gliadin ration made it adequate for growth. Hence the failure to grow when gliadin is the sole protein in an otherwise adequate diet is a specific stunting due to shortage of lysine.

When growth is retarded by inadequate intake of this particular amino acid, the emaciated appearance characteristic of animals attempting to grow on an insufficient energy intake is not to be expected. Osborne and Mendel have recorded numerous cases of suspension of growth of young rats, especially when kept on rations

containing gliadin as a sole protein food. Here the inadequacy of the lysine intake results in retardation or even complete suspension of growth, but the animal may remain quite healthy and symmetrical. Moreover rats may be subjected to this type of stunting for a remarkably long time (even as long as would normally cover the entire growth period) and still retain their capacity to grow when given an adequate diet.

In contrast to the striking extent to which lysine deficiency may result in simple suspension of growth without apparent injury, it has repeatedly been shown that a tryptophane deficiency is much more speedily disastrous; and the experiments of Rose and Cox indicate that this must also be anticipated in case of a deficiency of histidine; while the cystine stunting described by Woods, like the lysine stunting of Osborne and Mendel, resulted in retardation or suspension of growth with no evidence of a pathological condition in the body. Hence it is plain that the results of protein stunting may be different according to the particular amino acid shortage which is involved. In general, however, the evidence indicates that stunting from shortage of protein is apt to involve less injury than other forms of stunting.

### *Mineral Elements*

Certain mineral elements have long been recognized as playing an important part in growth and development. Infants (and young mammals generally) are born with a reserve store of iron usually sufficient to prevent any danger of iron deficiency up to about the end of the normal suckling period. At any time after this initial reserve supply has been used, the iron in the body will be found very largely localized in the blood. The blood usually constitutes less than 7 per cent of the weight of the body but contains more than 70 per cent of its iron content. Hence a deficit of iron becomes more noticeable in the blood than in the other tissues — growth may not cease but the child (or young animal) may grow anemic.

To an even greater extent than the iron is localized in the blood, the calcium of the body is localized in the bones and teeth. The skeletal system contains over 99 per cent of the body calcium. A low intake of calcium during growth retards the development and calcification of the bones and teeth. Such development may also be interfered with by inadequacy of the phosphorus supply,

especially at early ages when the muscles are growing rapidly and competing with the bones for the phosphorus which the blood brings. Thus the low-phosphorus type of rickets appears to be more common than the low-calcium type; but considering the period of growth as a whole, there is greater danger of shortage of calcium than of phosphorus. Several investigators, in studying the effect of diet upon growth of bone, have found that the bones formed in a young animal kept on a diet poor in phosphorus and calcium are apt to be soft, spongy, and weak, and that this may be prevented by the simple addition of calcium phosphate to the food. Confidence in the value of the antirachitic vitamin must not lead to any lack of care in providing a liberal supply of calcium and phosphorus during growth. For, whatever aid we may secure, through vitamins or otherwise, in maintaining a favorable condition for growth of bone, calcium and phosphorus are the chief elements which must always be supplied and retained in liberal amounts as actual constituents of the skeletal framework of the body.

Because the normal calcification of children's bones often lags behind other phases of their bodily development, much experimentation has been devoted to the problem of the relations of age, growth, and food to the body's increase of its percentage of calcium. Calcium-balance experiments with children, from which their increases of body calcium percentage can be calculated, have been considered in Chapter XIV. As another means of study of the calcium retention, extensive series of analyses have been made of rats of known hereditary and nutritional history, which have been differently fed under controlled conditions, or which have made different rates of growth on the same diet. In such experiments by Briwa, by Conner and Kao, and by Lanford and Campbell in the writer's laboratory, it has been found that neither more rapid growth as an individual characteristic nor the increase of the growth rate by enrichment of the dietary in its protein content augments the rate of increase of percentage of calcium in the body; but that this is augmented by enrichment in the calcium content of the diet (in experiments with rats, up to about 0.6–0.8 per cent of calcium in the dry weight of the food).

The findings outlined in the two preceding paragraphs illustrate the distinction emphasized by Todd between growth, on the one hand, and, on the other hand, development of the maturation



pattern of the skeletal tissue with increase in the *percentage* of bone mineral which it contains. (We cannot here pursue the further point that there need not always be exact parallelism between calcium percentage and the details of the Roentgenograms.)

In contrast with the purely individual differences and those resulting from an increased percentage of protein in the food, are the results of increasing the calcium content of the diet.

By analyses of relatively large numbers at each of several ages, it has been found that rats of families on food of optimal calcium content (0.64 to 0.80 per cent of the dry food mixture) attain at an average of one month of age a percentage of body calcium which is attained only at an age of four to five months by their cousins of families whose food is of only about minimal-adequate calcium content, *i.e.*, 0.20 per cent of calcium in the dry food mixture (Lanford, Campbell, and Sherman, 1941).

Moreover, the families living upon the more liberal calcium level showed higher vitality and lower death-rates at all stages of the life cycle. During the decades in which artificially refined foods were increasingly used and the importance of the protective foods had not yet become known, calcium intakes have doubtless very often been suboptimal. Readjustment under the guidance of the newer knowledge of nutrition is now in progress; but even recent calcium-balance studies with American children indicate that an undue proportion of our child population is apt to lag in this phase of normal development, and that continued emphasis upon the importance of greater prominence of calcium-rich food is still needed in many if not most American communities.

### *Vitamins*

In the chapters dealing respectively with thiamin, riboflavin, vitamin A, and the vitamins D, we have considered the influence of these factors upon growth and development. The reader is therefore already aware that while these are all "growth factors" the relation of each to the process of growth and development is different.

As these have been discussed in such recent chapters, space need not be taken to review them here; and, as already pointed out, any attempt to generalize for vitamins as a group would be misleading, because these substances are not a natural group from either the chemical or the physiological point of view.

### Applications and Interrelations

In applying the newer knowledge of nutrition to the practical problems of the feeding of children we should always give the child the benefit of any scientific doubt; and this may demand, among other things, the investment of much time and thought in the patient and persistent education of the child into good food habits. Where differences of interpretation or emphasis appear in the scientific literature of the subject, as, for instance, in the greater emphasis upon milk by some writers and upon fruit juices by others, it is well to give the child the benefit of the doubt by feeding liberal allowances of both milk and fruit juice, even though it takes time and trouble to establish the habit of the consumption of the desired liberal amount of milk.

McCollum has conducted a controlled experiment in an orphanage and found that the addition of a quart of milk a day to the diet of each of 42 children resulted in improvements in their rate of growth and in their general well-being, quite as indicated by experimentation upon laboratory animals. Here the original diet may have been visibly in need of improvement even without experimental demonstration; but in the investigation conducted by Corry Mann the starting point was a dietary which was already good.

Mann (1926) worked during a period of four years in an English institution for boys. The boys were between 6 and 11 years old. They were divided into seven groups, carefully matched as to age and size. The first group received the regular diet "originally chosen with every regard for the welfare of the children to be reared upon it"; the second group was given, in addition, one pint of milk per capita daily; the third, sugar equivalent in calories to the pint of milk; the fourth, butter from grass-fed cows in such amount as to furnish the same number of calories as the milk; the fifth, an equivalent amount of vegetable margarine; the sixth, sixty-five Calories of edible casein; and the seventh, three fourths of an ounce of fresh watercress per day. The control group gained, during the four-year period, 3.85 pounds and 1.84 inches each; the milk group made the largest gains of all, viz. 6.98 pounds and 2.63 inches; the butter group came second with 6.3 pounds and 2.2 inches; and the watercress group came third, indicating that extra vitamin (especially vitamin A) was more important in these

cases than extra calories or extra protein even when the latter was of such excellent a kind as casein. Sir Walter M. Fletcher, eminent English physician and member of the British Medical Research Council, emphasized strongly the significance of this work as showing that even on a dietary "medically adjudged to be sufficient for healthy development, the boys were in fact not attaining to the physical and mental growth of which they had the potentiality, and to which they did attain when given an extra daily ration of milk." And he pleaded that no other improvement, however desirable in itself, should be given priority over better nutrition: "First things should come first."

Orr\* and Leighton and Clark† have shown on a still larger scale that addition of milk to the diet of school children has a markedly larger influence upon growth in both height and weight than does the addition of an equal number of calories in such other form as biscuit; and also that the children getting the additional milk showed a superiority of "condition" which is not readily defined, but is recognizable as standing for something beyond pounds and inches; and also these children showed greater alertness and buoyancy of spirits.

It is important to recognize that even if the better feeding can not be permanent, it yet may confer a permanent benefit.

In a conference looking to nutritional improvement of the children of Puerto Rico, the question was raised whether it would be wise to introduce milk into the school lunch when it was not certain that the milk supply would continue. "Yes," rightly said Dr. McKinley, "for every day that the child *does* get the milk, he puts calcium and vitamin A in his bank."

Moreover, the material "banked" is not simply laid by as a passive reserve. In putting the body forward in development it becomes a permanent addition to the bodily "working capital," as in the better bone development observed by MacNair (1939) to result from the addition of codliver oil to even a diet which appeared to be good. Both vitamins A and D probably contributed to this improvement.

Boyd, Drain, and Nelson among others have shown that an increased use of milk, fruit, and vegetables in the dietaries of children is followed by a great reduction in the occurrence and severity of dental caries; though Bunting questions whether this un-

\* *Lancet* 1928, I, 202.

† *Lancet* 1929, I, 40.

doubted effect of food is to be interpreted on nutritional or on bacteriological grounds.

Boyd, Drain, and Stearns (1933) have further confirmed the fact that human dental caries is in general (though perhaps not in every individual case) related to the food; and they find a definite and significant relation between the level of retention of calcium and phosphorus and the resistance of the teeth to decay. They emphasize the view that for optimal results there is need of higher calcium retention than has usually been accepted as adequate.

Any of the many aspects of growth and development may be influenced by seasonal relationships. Temperatures which increase energy metabolism, either directly or through encouraging more exercise and higher tonus of the muscles, naturally induce the eating of a larger amount of food.

Benedict and MacLeod found that the heat production of rats was distinctly higher at 25.7° C. than at 28.9° C., the "critical temperature" for this species being apparently about 28° C. Correspondingly the heat production averaged 10 to 12 per cent lower in summer than in winter. In harmony with this is the observation of Campbell that food intake expressed in calories per gram of rat per day is lower in summer than in winter. And in this connection it is also of interest that Gloy found higher growth rates (among these rats) in winter than in summer. Eating more food to supply their greater calorie need in winter they thereby increase their intake of proteins, mineral elements, and vitamins.

Similarly children's appetites and energy requirements may be increased by exercise and, with the diet well balanced, this will mean, along with the increased intake of calories, a correspondingly increased intake of all the growth essentials. Hence exercise and good feeding act together in promoting the development of the child.

### **Growth Experiments as a Means of Research**

Previous chapters have afforded illustrations of the use of growth experiments as means of investigation in many different branches or aspects of the chemistry of food and nutrition. So widely is this means of research now used, that it has become important to consider with some care the quantitative applicability of the usual statistical interpretation of the experimental data thus obtained.

Here the primary question is, Will the variations in data of like feeding experiments show a normal frequency distribution, *i.e.*, when plotted approximate a symmetrical bell-shaped curve, so that we may justifiably feel confident in applying the usual quantitative statistical interpretation to such data?

Since the work of Rietz and Mitchell (1908) this has seemed sufficiently probable to warrant the use of the ordinary method of

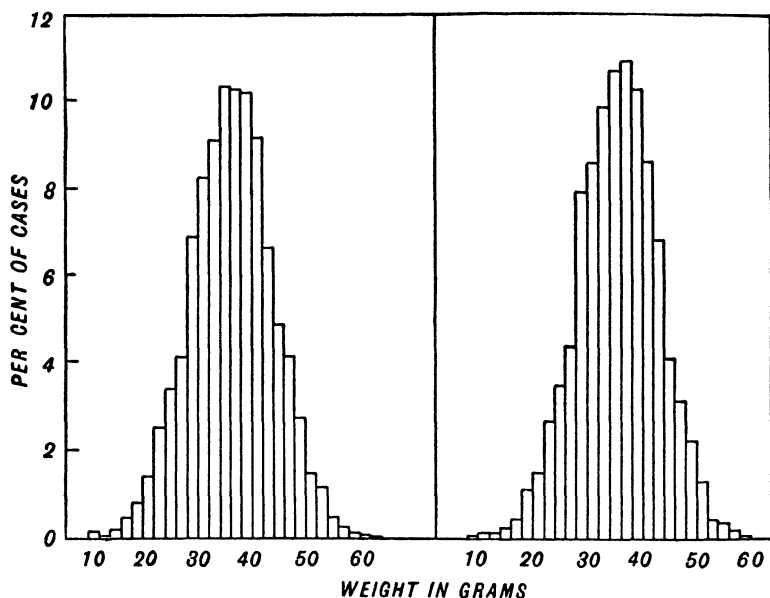


FIG. 44. — Frequency diagram for weights of rats, of same hereditary and nutritional history, at 28 days of age. On the left, data of 3092 males, skewness  $-0.04$ ; on the right, data of 3243 females, skewness  $-0.04$ . (Courtesy of the *Proceedings of the National Academy of Sciences*.)

handling such data. There are now at hand extended series of measurements of growth which actually demonstrate a very satisfactory symmetry in the frequency distributions, as shown in Fig. 44, and justify a high degree of confidence in the use of the classical methods of simple statistical interpretation, such as the computation of probable errors of the mean result of a series and of the difference between the means of two series, and the numerical expression of the probability or “chances” that an observed difference is statistically significant (Sherman and Campbell, 1934).

Baldwin (1925) found a parallelism of mental and physical development in the superior children that he studied.

The journal of the American Medical Association has said editorially: "In the absence of more definite correlation between the psychic and the physical, it is well to strive for ideal growth of the body and thus provide the most favorable sphere for the development of the mind."

But also, as other editorials in the same Journal have pointed out, especially in commenting upon the work of Wetzel (1932-1934), ideal growth is not necessarily the most rapid growth that can be induced. Rather it is the growth-rate induced by the plan of feeding which yields the best results for the life-cycle as a whole.

Certain first-findings of Cornell and Columbia investigators, respectively, which on superficial view may seem to differ in their trend, are not to be interpreted as discordant but rather as illustrating the complexity and importance of this field of nutritional research, as will be explained more fully in a later chapter.

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## CHAPTER XXVII

### DIETARY STANDARDS, THE DIFFERING RATIOS OF ADEQUATE TO OPTIMAL INTAKE, AND THE PRINCIPLE OF NATURAL WHOLE

#### The Concept of Dietary Standards as Modified by Recent Advances of Nutritional Knowledge

DURING the latter half of the nineteenth century, the teachings of Liebig, of Lawes and Gilbert, of S. W. Johnson, and other leaders were becoming widely effective in the more scientific feeding of farm animals. The general concept was developed that the nutritional needs of any animal species could be determined with due reference to age, size, and activity; and expressed in terms of the nutrients found by conventional food analysis. Simple arithmetic should then (it was thought) suffice to enable the farmer to use his crops or purchased materials in feeding his animals to his best economic advantage, taking account of the amounts of nutrients needed for the optimal growth and productivity of his animals, and the compositions and costs of available food materials.

For about the space of a generation centering on the turn of the century, the same idea was actively developed in relation to human nutrition. In America this development was largely under the leadership of Atwater, who was, however, careful to point out that *a dietary standard is only an indication, not a rule*. Having enjoyed the privilege of close collaboration with Atwater in the years in which he was formulating his most mature views, the present writer may emphasize the fact that had the experimental evidence of the more recent years been available then, Atwater would undoubtedly have emphasized still more strongly the limitations of so-called dietary standards and the fact that nutrition can be standardized or brought into equilibrium at different levels.

*The purpose of the present chapter* is to indicate how the recent advances of nutritional knowledge call for increased discrimination in the use of dietary standards, how standards in terms of

relative amounts of the different types of foods ("distribution standards") may be as significant as the standards which have been formulated in terms of the nutrients revealed by chemical analysis, and how any such standard or combination of standards needs to be used with scientific discrimination. What is helpful for certain practical purposes of dietetics and food economics, may yet be too dogmatic or oversimplified to be used directly in the expression of scientific findings without danger of ambiguity.

Laboratory measurements of the different phases of metabolism, under known conditions of age, sex, size, and occupation, have afforded widely useful indications as to the food-needs of normal people. But with growing knowledge, especially when the observations made directly upon human subjects are supplemented by studies extending throughout the natural life-cycles and successive generations of laboratory animals, it has been found that the relation between the minimal normal or merely adequate and the optimal level of intake is not the same for all factors of our nutrition.

In the case of total food calories the optimal intake at any given age and size is very nearly the same as the intake demonstrably necessary to support the desired activity and gain of weight in a child, or the desired degree of fatness in an adult of stated size, sex, and activity. Any considerably higher level of food-calorie intake leads either to digestive difficulty or to undue fatness. The best results are those which follow the eating of only about so many food calories as are demonstrably needed to maintain the *status quo* of the healthy adult or the normal combination of activity and growth in the child.

In the case of protein, some students of nutrition reason in the same way as for energy intake, while others believe that a liberal margin, even as much as twice the minimal amount demonstrably needed, may be advantageous. As explained in Chapter XI, an up-to-date discussion of the long-standing problem of high *vs.* low protein should logically take account of each amino acid individually; and such a discussion could reach no really conclusive and practical outcome until more is known *quantitatively* of the occurrence and functions of individual amino acids. Meanwhile the great majority of those having to prescribe or teach levels of protein intake are reasonably satisfied to use as a so-called standard (indication or guide might be a better word) an allowance 50 per

cent higher than the average of laboratory measurements of actual requirements in the sense explained in Chapter XI.

For some time it has also been customary to apply the same percentage margin over measured minimal needs in setting "standard" allowances for dietary intakes of phosphorus, of iron, and of calcium. In view of the ways in which a large proportion of the phosphorus and much the greater part of the iron in the body exist and function, as well as the trend of evidence of quantitative feeding experiments thus far, this is perhaps about as satisfactory for phosphorus and iron as for protein; but we now see a somewhat different relationship in the case of calcium. Much the greatest part of the body calcium is in a relatively insoluble form in the bones and teeth. The daily exchange of calcium in the body *may* therefore be relatively low, — equilibrium being establishable on a considerably lower intake of calcium than of phosphorus, — but the evidence of recent and very extensive experiments now indicates (Chapters XIV and XXVI) that for best permanent results the level of intake of calcium should be at least twice the minimal-adequate level. Thus a dietary allowance which aims to make possible the full realization of innate potentialities, will call for a much larger margin over mere minimal adequacy in the case of calcium than in the case of protein or, probably, of iron or phosphorus. The conventional grouping of mineral elements for convenience of subdivision of the subject matter of nutrition should not be allowed to obscure the fact that these elements may differ greatly among themselves as to ratios of adequate to optimal intake.

And among the vitamins we may expect equally large if not still larger differences in this respect. Each should be studied entirely on its own merits. For riboflavin and vitamin A, evidence has been cited in Chapters XIX and XXII that the optimal allowance would probably be at least three or four times the minimal-adequate dietary level; and from less direct evidence it seems probable that similarly liberal intakes of vitamin C may also be well justified. But it would not be scientific to assume such a wide difference between the minimal-adequate and the optimal intake or desirable dietary allowance for every vitamin. We need studies of each vitamin separately, by the methods adapted to the individual case, and with no presumption that what has been found for any one vitamin should logically hold true for any other one.



### The Problem of Factors as Yet Unmeasured and the Principle of Natural and Nutritional Wholes

As our present list of nutritionally essential factors may, perhaps, not yet be qualitatively complete, and certainly the quantitative need for some individual factors has not yet been measured, how much confidence can we have in dietary standards constructed on the basis of our present knowledge?

Here it is helpful to consider the general principle of *natural wholes*.<sup>\*</sup> An organism is more than a mere summation of its parts, it is an *organized* whole and functions as such in nature. Many of nature's wholes have evolved under the influence of each other, and so bear relationships to each other which have been more or less fixed by the evolutionary process. Hence, while it may be true that if we tried to subsist upon mixtures of the known chemical essentials in artificially purified forms we should probably sooner or later find ourselves inadequately nourished for lack of something still unknown, it is also true that, if instead of artificially purified foodstuffs we use natural foods such as have constituted a part of the environment in which our bodies have evolved and to which they are nutritionally adjusted, then any essentials which may be still unknown are consumed as they occur in the natural wholes which we eat.

However, it is something of a scientific safeguard of the well-balanced nutritional value of a dietary, as well as a training-exercise in dietary calculation, to figure individual dietaries and community food supplies in terms of a set of quantitative requirements or standards.

The greater the number of factors thus quantitatively ensured the fewer the possibilities of inadequacy at any other point; and the more nearly natural our food, the less is the danger of shortage of any nutritional factor, known or unknown.

### Desirability of Two Types of Standards

The suggestion has been made by Simmonds and endorsed editorially by the *Journal of the American Medical Association*, that at the present stage of development and dissemination of nutri-

<sup>\*</sup> The principle of natural wholes has been developed philosophically by Smuts under the term "holism" in his book *Holism and Evolution*, and in an article on holism in the *Encyclopedia Britannica*.

tional knowledge there is "too much of a tendency" to discuss nutrition in terms of the individual chemical factors involved, and that there is more to be gained through discussion of balanced nutrition in terms of the articles or types of food which one actually chooses and consumes.

Our present purpose is not so much to debate the relative merits of the two plans as to illustrate both, and invite attention to the value of each. Unquestionably, the student of the chemistry of nutrition should be prepared to think in terms of *both* the standards set up in terms of individual nutrient factors *and* the distribution standards such as are illustrated in Tables 53 and 54 (toward the end of this chapter) which are set up in terms of advice as to how the needed food calories shall be drawn from (or distributed over, or apportioned or allocated among) the different articles or types of food.

As research lengthens the list of chemical factors known to be essential to our nutrition, it becomes increasingly more practical (instead of setting a quantitative standard for each known chemical essential) to use the sort of standard which calls for so many total food Calories, of which not less than certain percentages shall be in the form of certain articles or types of food.

If it be desired to simplify to the point of escaping all need to deal with percentages, one may say not less than so much of this, and not less than so much of that, in the day's dietary.

The great disadvantage of this latter plan is that, except on a few points, the science of nutrition best gives guidance to dietetics in terms of general types rather than individual articles of food, leaving individual freedom of choice within the general groups or types; but foods of the same type may be marketed with such widely different moisture content (fresh or dried peas or peaches, for example) that mere weights or measures are too far from having a definite quantitative scientific significance.

Our long-range objective should be to realize the complexity of the problem, the interrelations of the numerous factors, and the need of a many-sided view; and yet be not discouraged, by our difficulties and limitations, from the effort to put our knowledge of nutrition and its professional application upon as quantitatively accurate a basis as is possible.

In the present state of nutritional knowledge, the most soundly scientific procedure seems to be to put dietary standards only

partly in numerical terms for chemical factors, and make at least equal use of distribution standards and other simplifications in terms of everyday foods.

Another simplification takes the form, not of omitting consideration of the conventional standards of requirements for individual nutrient factors at different ages, but of working through them to a concept of what constitutes a well balanced dietary for normal human nutrition generally. Rose has made much use of this general plan in her teachings and writings; using for its practical expression the "share" system described and fully illustrated in her *Foundations of Nutrition*, Third Edition (1938).

The ultimate simplification is that of Lord Astor, chairman of the League of Nations' mixed committee on nutrition. His standard is: *Enough food*, including enough of the *right kinds* of food.

### What Constitutes Enough Food?

Stated in the least technical terms, "enough" food means a sufficient amount to satisfy hunger, to support one's work, and to maintain the best body weight; and science has amply established the fact that it is *total food Calories* which primarily corresponds to this concept of *enough*. In scientific studies and formulations, and sometimes for special reasons in everyday practice, we "count the calories": more often, for the purposes of personal guidance it suffices to "watch the weight."

Some decades ago, Langworthy showed that the best available quantitative records of food consumption by different races and in different parts of the world indicates very similar total calorie needs for people of like age, size, and activity everywhere.

The world-wide studies of basal metabolism which have since been made have been largely discussed in terms of racial factors and climatic influences; but are especially noteworthy as revealing that the factors just mentioned have such slight effects, when any, and that the energy requirements of people of like age, size, and activity are so nearly the same everywhere.

There is also a greater degree of quantitative mutual confirmation between the earlier and the more recent data than appears upon the surface, and the present chapter seems the appropriate point at which to suggest such further consideration of this aspect as may appeal to the individual reader, or be deemed significant for a course of study. Langworthy like Atwater, and he like Voit,

expressed data of food consumption on the basis of that of the then current concept of the "average man," meaning a man of average size and of full muscular development engaged for the then average day of perhaps ten working hours upon manual labor of a moderately heavy kind. For such a man the approximate finding and resulting round figure "allowance" or "standard" was 3000 Calories of food actually eaten (consumed in the strict physiological sense), while to provide a margin for losses and shrinkages of various kinds, the standard for such a man's food consumption in the economic sense was often placed about ten per cent higher. Sometimes, too, the allowance was made still more generous as recognition or encouragement of an ideal of more energetic work. Partly on this principle and partly as a sort of bonus, army rations often provide more total calories than are actually eaten. In such ways as these a scientific net estimate of 3000 Calories for the above-mentioned "average man" may be increased to from 3300 to 3500 for reasons of "policy" and such a situation has often given the impression of greater differences of expert opinion than actually exist.

In the sense of actual nutritional need, there is now (as we saw in Chapters VIII to X) a large volume of consistent evidence as to energy requirements; and correspondingly there is a well established consensus of scientific opinion in support of a round-figure standard of 3000 Calories a day for the above-described "average" man (with more than present-day average activity) or an approximately corresponding standard of 2400 Calories per capita of the adult population.

What might superficially appear as a difference of 20 per cent in the figures just given is only an apparent difference, the result of the use of what now seems a more practical basis of expression, and does not mean either change or conflict of scientific opinion as to the energy needs of human nutrition.

For several reasons the lower and broader basis of expression now seems more useful in practice. As the general emancipation of men from the heavier forms of muscular work by the use of machinery and the shortening of working hours combine to decrease the percentage of men whose work is muscularly as heavy as that of the average man of the past, the older "average man" figure becomes increasingly conventional; but should not mislead the scientific student.

The increasingly used round figure of 2400 Calories per adult person is the same as the conventional former allowance for an average woman figured as needing four-fifths as much food as the 3000-Calorie man.

### **Significance of Data of Per Capita Food Consumption**

At first thought it might seem that in a study in which we are seeking to advance nutrition into the ranks of the exact sciences, all emphasis in a discussion of needs and standards would emphasize the differences due to age and size, and that any consideration of per-capita data would be a backward step. Such a judgment might, however, lack sufficient discrimination.

Data of actual food consumption, for instance, can be kept objective so long as we keep them to the per-capita or described-group basis. That such-and-such people ate such-and-such food is an entirely objective statement of fact. Considered with scientific discrimination, a food-consumption record begins to lose objectivity as soon as we begin to express it upon any basis which introduces an assumption or opinion as to how different people compare with each other in respect to the amounts that they eat or need.

For instance, if we are informed that the food supply of a given community provides 0.9 gram of calcium per capita per day, the information is objective and perfectly clear so far as it goes; while if told that the calcium content of the food supply is barely up to standard considering the proportion of children in the population, we see that the objective facts have been combined with subjective judgments, and we must ask: What standard? And how does one know just how the calcium requirements of children and adults compare?

### **Energy Allowances for Adults**

It has been shown that different normal individuals of similar age and physique show a rather striking similarity in their energy requirement when performing equivalent amounts of muscular work, and that it is primarily the muscular activity, and only in minor degree personal idiosyncrasy or the amount of food eaten, which determines the amount of energy transformed in the body.

A dietary standard of high fuel value, and designed to maintain metabolism on a high energy level, provides, therefore, pri-

marily for a large amount of muscular work. If this work is not performed and the food continues to be eaten and digested, we may expect to find a storage of fuel in the body chiefly in the form of fat, and this is true whether the surplus food eaten is carbohydrate, fat, or protein, — though to a less degree in the case of protein because of its specific dynamic action.

Thus the store of body fat which a person carries is the most reliable indication as to whether the amount of food habitually eaten is or is not properly adjusted to the work performed. The storage of fat does, however, in itself modify the food requirement. While it is true, as has been shown, that, as between a lean and a fat man having the same weight, the lean man will have the greater food requirement, yet it is also true that when any given man becomes fat, his increased size of body calls for increased metabolism of energy. The work involved in walking, for example, will increase in proportion to the weight moved (*i.e.*, to the weight of the body as a whole); and the work of respiration will increase with the weight of that part of the body which must be moved with the expansion and contraction of the lungs; while if fat is deposited in such a way as to interfere directly with the free play of the muscles, there may be an actual lowering of muscular efficiency, so that a larger expenditure of energy may be required in order to produce a given amount of work. If the high energy intake is continued and the digestion remains normal, the storage of fat will continue until it raises the energy expenditure of the body to a point where the food is no longer in excess for the maintenance of weight at the higher level. If the store of fat carried when this point is reached is excessive, the fuel value has been too high; if the store of fat is not excessive, the fuel value of the diet, although greater than would have been necessary to maintain the body at its former weight, has not been too high, and the body has acquired an asset whose utility may not always be recognized, but which may be of great value in case of accident, illness, or exposure. For body fat functions in various ways: as a fuel reserve; as a protection from chill, shocks, or bruises; and as a packing to prevent accidental shifting of position of the kidneys and possibly other organs.

From a study of the records of body weight in relation to the mortality records as collected by life insurance companies in the United States and Canada, it is found that among young

people the greatest vitality coincides with a weight somewhat above the generally accepted averages, while with middle-aged and elderly people a condition of less than average fatness is most favorable to vitality and longevity. Another way of stating the same facts is: That the average of healthy men and women keep themselves slightly too thin while young, and allow themselves to grow slightly too stout as they grow older.

*The optimal fatness*, as indicated by these statistics, is that which corresponds to the individual's height and to age 30 in the so-called standard tables.

The data here reproduced (Table 51) are those of the Life Extension Institute. The height includes ordinary shoes and the weight includes ordinary indoor clothing.

TABLE 51. — HEIGHT-WEIGHT-AGE RELATIONS (Life Extension Institute)

TABLE OF HEIGHTS AND WEIGHTS BASED UPON THE REPORT OF THE MEDICO-ACTUARIAL INVESTIGATION, 1912, COVERING AN ANALYSIS OF 221,819 MEN AND 136,504 WOMEN

TABLE OF AVERAGE HEIGHTS AND WEIGHTS—MEN

| Age | 0 in. | 1 in. | 2 in. | 3 in. | 4 in. | 5 in. | 6 in. | 7 in. | 8 in. | 9 in. | 10 in. | 11 in. | 0 in. | 1 in. | 2 in. | 3 in. | 4 in. | 5 in. |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|-------|-------|-------|-------|-------|-------|
| 15  | 107   | 109   | 112   | 115   | 118   | 122   | 126   | 130   | 134   | 138   | 142    | 147    | 152   | 157   | 162   | 167   | 172   | 177   |
| 20  | 117   | 119   | 122   | 125   | 128   | 132   | 136   | 140   | 144   | 148   | 152    | 156    | 161   | 166   | 171   | 176   | 181   | 186   |
| 25  | 122   | 124   | 126   | 129   | 133   | 137   | 141   | 145   | 149   | 153   | 157    | 162    | 167   | 173   | 179   | 184   | 189   | 194   |
| 30  | 126   | 128   | 130   | 133   | 136   | 140   | 144   | 148   | 152   | 156   | 161    | 166    | 172   | 178   | 184   | 190   | 196   | 201   |
| 35  | 128   | 130   | 132   | 135   | 138   | 142   | 146   | 150   | 155   | 160   | 165    | 170    | 176   | 182   | 189   | 195   | 201   | 207   |
| 40  | 131   | 133   | 135   | 138   | 141   | 145   | 149   | 153   | 158   | 163   | 168    | 174    | 180   | 186   | 193   | 200   | 206   | 212   |
| 45  | 133   | 135   | 137   | 140   | 143   | 147   | 151   | 155   | 160   | 165   | 170    | 176    | 182   | 188   | 195   | 202   | 209   | 215   |
| 50  | 134   | 136   | 138   | 141   | 144   | 148   | 152   | 156   | 161   | 166   | 171    | 177    | 183   | 190   | 197   | 204   | 211   | 217   |
| 55  | 135   | 137   | 139   | 142   | 145   | 149   | 153   | 158   | 163   | 168   | 173    | 178    | 184   | 191   | 198   | 205   | 212   | 219   |

TABLE OF AVERAGE HEIGHTS AND WEIGHTS—WOMEN

| Age | 8 in. | 9 in. | 10 in. | 11 in. | 0 in. | 1 in. | 2 in. | 3 in. | 4 in. | 5 in. | 6 in. | 7 in. | 8 in. | 9 in. | 10 in. | 11 in. | 0 in. |
|-----|-------|-------|--------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|-------|
| 15  | 101   | 103   | 105    | 106    | 107   | 109   | 112   | 115   | 118   | 122   | 126   | 130   | 134   | 138   | 142    | 147    | 152   |
| 20  | 106   | 108   | 110    | 112    | 114   | 116   | 119   | 122   | 125   | 128   | 132   | 136   | 140   | 143   | 147    | 151    | 156   |
| 25  | 109   | 111   | 113    | 115    | 117   | 119   | 121   | 124   | 128   | 131   | 135   | 139   | 143   | 147   | 151    | 154    | 158   |
| 30  | 112   | 114   | 116    | 118    | 120   | 122   | 124   | 127   | 131   | 134   | 138   | 142   | 146   | 150   | 154    | 157    | 161   |
| 35  | 115   | 117   | 119    | 121    | 123   | 125   | 127   | 130   | 134   | 138   | 142   | 146   | 150   | 154   | 157    | 160    | 163   |
| 40  | 119   | 121   | 123    | 125    | 127   | 129   | 132   | 135   | 138   | 142   | 146   | 150   | 154   | 158   | 161    | 164    | 167   |
| 45  | 122   | 124   | 126    | 128    | 130   | 132   | 135   | 138   | 141   | 145   | 149   | 153   | 157   | 161   | 164    | 168    | 171   |
| 50  | 125   | 127   | 129    | 131    | 133   | 135   | 138   | 141   | 144   | 148   | 152   | 156   | 161   | 165   | 169    | 173    | 176   |
| 55  | 125   | 127   | 129    | 131    | 133   | 135   | 138   | 141   | 144   | 148   | 153   | 158   | 163   | 167   | 171    | 174    | 177   |

In the Keep-Well Leaflets of the Life Extension Institute from which Table 51 is quoted, the leading comments on under- and over-weight are as follows:

“Pronounced underweight before the age of 25 is an unfavor-

able condition, as it is often associated with lack of resistance to pulmonary affections and to other diseases of youth. After the age of 30, underweight, unless extreme, is not an unfavorable condition. Indeed, an investigation of the combined mortality experience of forty life insurance companies shows that the lowest mortality at middle life is found among those a few pounds under weight.

“When underweight is associated with a tendency to frequent colds and there is a condition of debility, anemia, or general malnutrition, it is, at any age, an unfavorable symptom, especially if there has been recent loss in weight. Under such conditions, dieting should be directed to improving nutrition and inducing a gain in weight.

“A moderate degree of overweight is well borne before the age of 35. After the age of 35, overweight is associated with an increasingly high death rate and at or after middle life it becomes a real menace to health, either by reason of its mere presence as a physical handicap or because of the faulty living habits that are often responsible for its development.”

### Energy Allowances for Children

As a result of compilation and study of data from dietary studies, nitrogen balance experiments, observations of the respiratory exchange, and direct measurements of energy output, the standards shown in Table 52 have been suggested.

Many attempts have been and still are being made to secure an easily applied and accurate measurement of the nutritional development of the child. Many of the references at the end of Chapter XXVI relate to this problem.

A normal rate of growth and development during childhood is considered more significant than close agreement with the average weight for height and age, for many moderate deviations from the average weight for height and age must undoubtedly be considered normal, according to individual build.

### Distribution Standards

M. S. Rose has developed a system of dietary standards for children which employs the principle, explained earlier in this chapter, of indicating what percentage of the total calories is to



TABLE 52. — FOOD ALLOWANCES FOR CHILDREN OF ABOUT AVERAGE WEIGHT FOR THEIR AGE. BASED ON GILLETT'S "FOOD ALLOWANCES FOR HEALTHY CHILDREN" AND ROSE'S "LABORATORY HANDBOOK FOR DIETETICS"

| AGE<br>Years | CALORIES PER DAY |           |
|--------------|------------------|-----------|
|              | Boys             | Girls     |
| 1            | 900-1200         | 800-1200  |
| 2            | 1100-1300        | 1000-1250 |
| 3            | 1100-1400        | 1050-1350 |
| 4            | 1200-1500        | 1150-1450 |
| 5            | 1300-1600        | 1200-1500 |
| 6            | 1500-1900        | 1450-1800 |
| 7            | 1600-2100        | 1500-1900 |
| 8            | 1700-2300        | 1600-2200 |
| 9            | 1900-2500        | 1800-2500 |
| 10           | 2100-2700        | 1900-2600 |
| 11           | 2100-2800        | 2000-2800 |
| 12           | 2300-3000        | 2100-3000 |
| 13           | 2500-3500        | 2300-3400 |
| 14           | 2600-3800        | 2400-3000 |
| 15           | 2700-4000        | 2400-2800 |
| 16           | 2700-4000        | 2250-2800 |
| 17           | 2800-4000        | 2250-2800 |

come from each chief kind or type of food. Table 53 shows such standards for children from 4 to 12 years of age, arranged in five age groups.

TABLE 53. — DISTRIBUTION OF CALORIES IN DIETS OF CHILDREN OF 4 TO 12 YEARS

| AGE IN<br>YEARS | PER CENT OF TOTAL CALORIES FROM EACH CLASS OF FOOD |       |                          |        |                         |  |
|-----------------|--|-------|--------------------------|--------|-------------------------|--|
|                 | Foods from<br>Cereal<br>Grains                     | Milk  | Vegetables<br>and Fruits | Fats * | Sugars<br>and<br>Sweets | Eggs,<br>Cheese,<br>Meat and<br>Other Flesh<br>Foods |
| 4-5             | 23-25  | 45-50 | 14-18                    | 5-8    | 2-5                     | 5-6  |
| 5-6             | 23-25  | 45-50 | 14-18                    | 5-8    | 2-5                     | 5-6  |
| 6-7             | 20-25  | 40-45 | 14-15                    | 10-12  | 3-4                     | 4-5  |
| 8-9             | 20-25  | 38-42 | 15-16                    | 12-13  | 4-6                     | 5-6  |
| 10-12           | 20-25  | 34-38 | 17-18                    | 13-14  | 6-8                     | 7-8  |

\* At least part of the fat to be butter or something known to furnish its equivalent of vitamin A.

Table 54 shows corresponding distribution standards to guide the planning of minimum cost and moderate cost dietaries, re-

spectively, for children of ages 5 to 16 years. The minimum cost standard is based on the recommendations of the Committee on Economic Standards of the New York Nutrition Council. The moderate cost standards are those used by Rose and Gray in judging the dietaries of child-caring institutions.

TABLE 54. — DISTRIBUTION OF CALORIES IN DIETS OF MINIMUM AND OF MODERATE COST FOR CHILDREN OF AGES 5 TO 16 YEARS

| FOOD GROUP                          | MINIMUM COST<br>DIET                  | MODERATE COST<br>DIET                 |
|-------------------------------------|---------------------------------------|---------------------------------------|
|                                     | <i>per cent of total<br/>Calories</i> | <i>per cent of total<br/>Calories</i> |
| I. Foods from cereal grains . . . . | 37                                    | 24                                    |
| II. Milk . . . . .                  | 22                                    | 32                                    |
| III. Vegetables and fruits          |                                       |                                       |
| A. Dried legumes . . . . .          | 3                                     | 1                                     |
| B. Other vegetables and fruits . .  | 13                                    | 16                                    |
| IV. Fats and oils . . . . .         | 14                                    | 12                                    |
| V. Sugars and sweets . . . . .      | 5                                     | 7                                     |
| VI. Meat, eggs, cheese . . . . .    | 6                                     | 8                                     |

*A simple distribution standard for adults* consists in the two-fold suggestion: (1) that at least half of the needed calories be taken in the form of fruits, vegetables, and milk (including cheese, cream, and ice cream); and (2) that at least half of whatever breadstuffs and cereals are eaten be taken in the "whole grain" or "dark" or "unskimmed" forms.

### Regarding Numerical Standards for Protein, Mineral Elements, and Vitamins

In the chapters dealing respectively with protein needs, with calcium and phosphorus, with iron, ascorbic acid, thiamin, riboflavin, and with vitamin A values, we have considered the problems of quantitative requirements and optimal intakes sufficiently to have covered there about all that we are prepared to say as to dietary standards for these factors, so that it does not seem necessary to discuss them further here.

Some of the considerations briefly sketched in the foregoing chapter are discussed further in other relationships in the chapters which follow.

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\* See also the references at the ends of the chapters which follow.

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## CHAPTER XXVIII

### CONSCIOUS CHEMICAL CONTROL OF THE INTERNAL ENVIRONMENT: THE PROBLEM OF THE BEST USE OF FOOD

#### Influence of Food Selection upon Internal Environment

FOR ABOUT a century until the most recent years, science has regarded the internal chemistry of the normal members of each species as being more rigidly specific than it really is.

As briefly mentioned in previous chapters, Liebig's investigations seemed to his contemporaries and to perhaps two generations of his successors to show that a plant or an animal will grow only so fast and so far as is consistent with the attainment and maintenance of the specific chemical composition of its kind.

Somewhat later than Liebig, another great scientist and influential teacher, Claude Bernard, gave cogent expression to essentially the same view in its more physiological aspect when he said that it is the *fixité* of the internal environment which enables an organism to cope with a new or changing external environment. The neat form of this generalization, and its usefulness in helping to explain the self-regulatory processes in the body and the spread of species over the surface of the earth, have resulted in its having been accepted and repeated somewhat dogmatically for two generations.

Meanwhile, however, there have developed physico-chemical principles, based on much more complete evidence, which call for revision of these over-simple postulates or first-approximations. We can no longer doubt that when different proportions of active factors are introduced into a system some difference in concentration levels or dynamic-equilibrium points must result. The postulated *fixité* of the body's internal environment can therefore be only approximately true; and, in its chemical aspects, must vary with the individual choice and use of foods. Normal people living on everyday foods introduce into their systems different

amounts and proportions of such active factors as are some of the mineral elements and vitamins, depending upon their choices among the staple foods and the relative proportions in which they consume them.

Until recently it could be thought (and doubtless it is still thought by many people of scientific training who have not sufficiently studied the recent evidence), that while what has just been said of concentration levels and equilibrium points is theoretically true yet practically the body's physiological regulation keeps its internal chemistry essentially constant. But as we saw in Chapter XVII, the recent analyses of blood plasma and of cerebrospinal fluid show that their concentration levels are significantly influenced by the levels of nutritional intake even in the case of so soluble and diffusible a substance as vitamin C. Similar relations are indicated, too, in the case of the fat-soluble vitamin A. Also, analyses of the body as a whole show (Chapters XIV and XXVI) that the calcium content of the body is significantly influenced by the calcium content of the food. And this is true not only as between adequate and inadequate intakes, but also as between different degrees of liberality of intake within the normal zone.

It may be recalled that by calcium balance experiments with children as well, it has been established that the rate of calcification in the normally developing body, and therefore the percentage of calcium in the body at a given age or size, may be varied by adjustment of the nutritional intake of calcium at a higher or a lower normal level, and that there are strong clinical indications that the higher normal levels lead to better human life-histories.

Full-life, successive-generation experiments with large numbers of laboratory animals (of species whose nutritional responses have been shown to be like those of human beings with respect to the nutrients under investigation) indicate that at least two other factors in our nutrition, — riboflavin (Chapter XIX) and vitamin A (Chapter XXII) — also show similar wide zones between the minimal adequate and the optimal levels of intake.

That riboflavin is a water-soluble, vitamin A a fat-soluble, and calcium in the form of bone mineral a relatively insoluble substance, shows that the principle of the flexibility of the body's internal environment and of its control through levels of nutri-

tional intake is applicable to substances of a wide range of properties.

While in the three cases just considered, the optimal levels of intake are relatively high, it is also to be noted that some other substance or element may be an essential factor in our nutrition and yet its optimal level may be in the lower or middle rather than the higher ranges of the normal zone. For calcium, riboflavin, and vitamin A are not "random samples" of nutrients; they were submitted to the long investigations briefly mentioned here because of the probability that among them might be found a chemical explanation of earlier nutritional research data (described in the section on adequate *vs.* optimal nutrition, beyond) in which the experimental variable was the quantitative relation between natural articles of food in the dietary.

The old questions, of what to eat and why, take on much new meaning in the light of the most recent evidence that daily food habit exerts a very significant influence upon the individual's internal chemistry, and has far-reaching effects upon his life history and his value to mankind.

### **The Practical Problem of the Best Use of Food**

The problem of the best use of food plainly presents both individual and community aspects. Food should be so chosen that the health and efficiency of the individual shall be served in the highest degree, and at the same time, it should be used with such regard to the economics of food supply that the possibility of optimal nutrition shall be brought within the reach of a steadily increasing proportion of the people, and shall finally be the birth-right of all.

As the preceding section has sought to show, the chemical aspect of the body's internal environment, — the chemistry of its tissues and fluids, — is more amenable to planned control and modification, and the possibilities of nutritional improvement of already normal health are therefore greater, than hitherto supposed.

How free are we to realize upon the option of a superior life process which this newer knowledge of nutrition offers? Are there other things to handicap us in our choice of food for superior nutritional well-being?

Both legislation and public opinion already demand that noth-

ing in the food supply shall be directly detrimental to health. And in general\* we need not fear direct injury from our daily food: we can and should think in the more optimistic and constructive terms of its possible significance for the advancement of our positive health.

Cases of food allergy, the hyper-sensitiveness which may make an ordinary staple food a source of distress or even of danger to some individual consumer, are probably not of very frequent occurrence when the population as a whole is considered. However important such phenomena may be to the individuals who suffer from them, they can hardly be regarded as of major importance in the general problem of the best use of food by the majority of people.

Of much broader and deeper significance than any of these possible handicaps is the problem of the choice of food for the best nutritional interests of the body; and whether this is accomplished with such conservation of the food supply as best to serve the community and the people as a whole. Certainly we should seek to make all dietaries adequate; but having found or attained the adequate we no longer rest content with that as if there were nothing more to be learned or accomplished. Not merely adequate but rather optimal nutrition is now the ideal: the consciously-held ultimate objective, whose descriptive definition is still a goal of nutritional research.

Already, however, we have convincing evidence of certain directions in which the typical American dietary can be improved.

### Nutritional Characteristics of the Chief Types of Foods

How best to draw upon the different types of food in meeting the nutritive requirement must always remain to some extent an individual problem, but the solution of the problem can be greatly facilitated by a consideration of the nutritional characteristics of our different foods.

Most of the common articles and types of food may be helpfully grouped according to their most significant nutritional characteristics as follows:

\* Problems of food adulteration are discussed in the author's *Food Products and Food and Health*.



(1) Breadstuffs and other grain products. Economical sources of energy and protein but not satisfactory in their calcium content or in their vitamin values. (If not too highly refined,\* however, grain products are an excellent source of thiamin.)

(2) Sugars and fats. Chiefly significant from the nutritional standpoint as supplementary sources of energy, although some fats are also important as sources of fat-soluble vitamins. (The small amount of fat as such, particularly of certain unsaturated fatty acids, which now appears to be specifically needed in nutrition is doubtless amply furnished by the milk, fruits, and vegetables of a normal food supply.)

(3) Meats (including fish and poultry). Rich in protein or fat or both, but showing, in general, about the same calcium and vitamin deficiencies as do the grains except that the meats contain more riboflavin and nicotinic acid.

(4) Fruits and vegetables. Greatly variable in their protein and energy values, but highly important as sources of mineral elements and vitamins.

(5) Milk. Important as source of energy, protein, mineral elements, and vitamins. The most efficient of all foods in making good the deficiencies of grain products and in ensuring the all-round adequacy of the diet.

It becomes apparent that a dietary made up, as so many American dietaries are, too largely of breadstuffs, meats, sweets, and fats, may be satisfying to the palate and to the traditional demand for variety, may furnish ample protein and calories with fats and carbohydrates in any desired proportions, and yet may fall far short of furnishing optimal amounts of some of the mineral elements and vitamins. We now understand how it is that liberal allowances of fruits, vegetables, and milk in its various forms, serve (in ways which until recently could not be fully appreciated) to make an ordinary dietary or food supply more conducive to optimal nutrition and health.

It will have been noticed that eggs were not included in the above characterizations of the chief types of food. In most respects, eggs may be regarded as intermediate between meat and milk. They will be mentioned again under the heading of the "protective" foods below.

\* Proposals for the restoration of thiamin to refined mill products are under active discussion at the time this is written.

### Food Values of American Dietsaries

The character of the average American dietary may be judged: (1) from the data of detailed and intensive dietary studies; (2) from the returns of food consumption studies made on a more extensive scale by less accurate methods; (3) from certain statistical returns of agriculture and commerce. As the findings of the three types of inquiry are sufficiently consistant, we here use figures obtained in the first way, as being fairly representative and as having been obtained by the method of highest precision. Calculations based on the data from 224 families, taken as typical and studied carefully by trained field workers from the United States Department of Agriculture and the New York Association for Improving the Condition of the Poor, show an average food value per man per day as follows:

|            |        |          |
|------------|--------|----------|
| Energy     | 3256.  | Calories |
| Protein    | 106.   | Grams    |
| Phosphorus | 1.63   | Grams    |
| Calcium    | 0.74   | Gram     |
| Iron       | 0.0179 | Gram     |

This series of 224 studies, while by no means the latest or most extensive inquiry into food consumption, is still a "benchmark" worthy of the careful attention of students of nutrition. More recent inquiries reported by Stiebeling and Coons (1939), Stiebeling and Phipard (1939), and others are listed at the end of the chapter. In a later section we summarize some of the findings of Stiebeling and coworkers.

Since it is well within the power of the consumer to alter the relative prominence of the different articles or types of food in the dietary or food supply, and indeed we often find considerable divergencies in the relative prominence of different foods in the dietsaries of neighboring families of similar economic status, it becomes important to consider what influence such variations in the food budget may be expected to have upon the nutritional character of the resulting dietary.

### Nutritional Significance of the Relative Prominence of Different Foods in the Dietary

From the results of numerous studies made by the United States Bureau of Labor Statistics, by the United States Depart-

ment of Agriculture, and by the New York Association for Improving the Condition of the Poor, it can be estimated that the typical American family spends from 25 to 40 per cent of its food money for meats and fish (including poultry and shellfish); about 5 per cent for eggs; about 10 per cent for milk, cream, and cheese; about 10 per cent for butter and other fats; from 15 to 20 per cent for bread and other cereal and bakery products; about 5 per cent for sugar and other sweets; about 15 per cent for fruits and vegetables; and about 5 per cent for miscellaneous foods and food adjuncts. This sketchy, "round figure" statement may be compared with the actual average of 224 typical American dietaries of some years ago shown in Table 55 herewith, and with the more elaborately analyzed data of recent studies shown in Table 59 (Chapter XXIX). At the same time it is plain that such a food budget, however prevalent, need not be regarded as fixed.

TABLE 55. — AVERAGE PERCENTAGE DISTRIBUTION OF COST AND NUTRIENTS IN 224 AMERICAN DIETARIES

| TYPE OF FOOD                    | RELATIVE COST | CALORIES | PROTEIN | PHOSPHORUS | CALCIUM | IRON  |
|---------------------------------|---------------|----------|---------|------------|---------|-------|
| Meat and fish . . . . .         | 32.19         | 18.99    | 35.34   | 26.36      | 3.86    | 30.37 |
| Eggs . . . . .                  | 5.47          | 1.77     | 4.64    | 4.02       | 3.64    | 6.25  |
| Milk and cheese . . . . .       | 10.59         | 8.08     | 11.56   | 20.61      | 55.76   | 5.11  |
| Butter and other fats . . . . . | 9.55          | 10.32    | .31     | .32        | .73     | .33   |
| Grain products . . . . .        | 18.29         | 38.20    | 37.25   | 30.27      | 15.67   | 25.87 |
| Sugar and molasses . . . . .    | 4.57          | 10.06    | .14     | .20        | 1.81    | 1.80  |
| Vegetables . . . . .            | 10.55         | 9.05     | 9.55    | 15.58      | 14.87   | 26.42 |
| Fruit . . . . .                 | 5.31          | 2.99     | .78     | 1.82       | 3.15    | 3.29  |
| Nuts . . . . .                  | .15           | .14      | .11     | .13        | .07     | .09   |
| Food adjuncts . . . . .         | 3.33          | .40      | .32     | .69        | .44     | .47   |

The 224 studies summarized in Table 55 represent a wide range of American conditions — urban and rural, well distributed over the country, over a number of years, and all seasons of the year, and including families showing a representative distribution of levels of income and of expenditure for food. The average of this entire group is, therefore, believed to present an essentially true picture of average American food economy. Furthermore, if this entire large composite "sample" (224 group-cases) be subdivided into a few large sub-groups according to the relative prominence of any one article or type of food, the differences appearing in the

average data of the large sub-groups thus obtained may be taken as a true indication of the nutritional outcome of such differences in choice of food as do actually exist within the range of typical normal conditions.

In order to find what differences of this kind normally exist in American food budgets and how they affect the food economy, certain conventional limits were employed to divide the 224 dietaries of Table 55 into three groups with reference to each type of food according as the place of this type of food was "low," "medium," or "high" in the dietary, as compared with average American practice. The results of a study of these data have been described in the last chapter of the writer's *Food Products*, Third Edition, and only the findings bearing most directly upon the immediate problem of the present chapter can be summarized here.

Grain products (including breadstuffs, cereals, etc.) are found to be most prominent in the dietaries of lowest cost. If the amounts of nutrients obtained *in proportion to cost* be considered, an increasing prominence of grain products in the food budget is found to give an increasing return in calories, protein, and phosphorus, while calcium and iron remain at about the same level. Iron can readily be increased by the use of a larger proportion of whole grain products in place of a part of the highly milled materials which are so prominent in most American dietaries; but even then, prominence of grain products in the dietary must in general be expected to lead to a relatively low calcium content, unless there is definite supplementation with calcium salts or high-calcium foods. From Table 55 above it will be seen that calcium is the only one of the factors of food value there measured of which the return from the grain products was less than proportional to the money cost. We should, however, also take account of the fact that an increasing prominence of grain products in the dietary tends to diminish the intake of vitamins A and C, and riboflavin. In order, then, that the consumer get the full benefit of the economy of the grain products as food without detriment to the nutritional value of the dietary as a whole, it is important that the breadstuffs and cereals be balanced by giving prominence to milk, vegetables, and fruits in order to assure ample supplies of calcium, of ascorbic acid, of riboflavin, and of vitamin A.

When the 224 dietaries here considered were divided into three groups according as the place of milk in the food budget was low,

medium, or high, it was found that an increase in the prominence of milk, from an average of 10.30 per cent of the total expenditure for food in the "medium," to an average of 19.38 per cent in the so-called "high milk" dietaries, resulted in an increase in all the outstanding factors of food value, with no increase in the total cost. Furthermore, the improvement was greatest at the point at which it was probably most needed, *i.e.*, in the calcium content of the dietary; and there was an undoubtedly very important increase in the riboflavin content and vitamin A value as well.

The cheapness of breadstuffs and the efficiency with which milk supplements them, give rise to the saying that "the dietary should be built around bread and milk."

Vegetables and fruit taken as a group may be ranked next after grain products and milk in importance as constituents of an economical and well-balanced diet. Like milk they tend to correct both the mineral and the vitamin deficiencies of the grain products; and in a sense they supplement the milk also in that many of the vegetables and fruits are rich in iron or vitamin C, or both.

The dietaries in which milk, vegetables, and fruit (together) were prominent averaged lower in cost and higher in energy, protein, phosphorus, calcium, and iron; and undoubtedly they also had higher vitamin values.

Based upon a suggestion made by Miss Gillett, the following simple budget for families who must buy all their food in city markets has been widely recommended and found useful:

Divide your food money into fifths —

One fifth, more or less, for vegetables and fruit;

One fifth, or more, for milk and cheese;

One fifth, or less, for meats, fish, and eggs;

One fifth, or more, for bread and cereals;

One fifth, or less, for fats, sugar, and other groceries and food adjuncts.

The recommendation that a fifth or more of the total food money be spent for bread and cereals aims at making a dietary more economical than the American average. In practice, the proportion spent for bread and cereals may well (and usually does) vary with the need for strict economy. It must usually be high in an extremely low-cost dietary and may be considerably lower where the level of expenditure is more liberal.

Whatever the level of expenditure, however, it seems wise to observe the two suggestions that:

(1) at least as much should be spent for milk (including cream and cheese if used) as for meats, poultry, and fish; and

(2) at least as much should be spent for fruits and vegetables as for meats, poultry, and fish.

These suggestions are said to have been found useful as a guide in both low-cost and liberal-cost food budgets and can obviously be applied in all cases in which even the simplest of records of expenditure are kept.

From among the 224 dietary records above discussed, 25 were taken at random and a calculation was made to see how their nutritive values would have been affected if, with no change in the amount of money spent or in the nature of the food selected, the quantities had been simply readjusted in accordance with the two suggestions just given. It was found that such readjustment without change in cost would leave the protein practically unchanged in amount while the calories and iron would be slightly increased and the calcium and phosphorus materially increased and brought into better quantitative relations with each other. Furthermore, the dietaries thus adjusted would undoubtedly be improved in their vitamin A and C values, and probably also in respect to riboflavin.

That progress in this direction is already taking place, gradually but upon a large scale, is shown by statistics of production, and railway transportation of different types of food; by comparative studies of urban food habits made at a 15-year interval by Miss Gillett; and by the more recent studies of Stiebeling and coworkers, mentioned above and briefly discussed in the section which follows.

### **Food-Consumption Studies of Stiebeling and Coworkers**

Space is not available here for any attempt at detailed consideration of the extensive food-consumption studies carried out by the United States Departments of Agriculture and of Labor, nor have the findings yet been published in full. The following notes on some of the points most directly connected with the subject-matter of this chapter may, however, be mentioned.

Stiebeling and Coons (1939) present in the form of a review article some of the outstanding trends, while Stiebeling and Phi

pard (1939) report in the form of a research bulletin the findings of large numbers of studies classified by geographic regions and by economic levels and accompanied with interpretive discussion. For the purpose of our present very brief mention we may draw upon the two publications without distinguishing between them.

Even among those who live upon farms, "the family's attitude toward home production" of food is still one of the major variables in the problem of planning for better nutrition. Among city families who must obtain their food entirely by purchase there is a corresponding difference of attitude which results in very different nutritional returns at the same economic levels. Thus among families spending about the same amounts for food in about equally favorable markets, some choose dietaries nutritionally rated as "good," while others get dietaries nutritionally only "fair," and still others buy dietaries nutritionally "poor," although spending as much for food as the neighbors whose dietaries were found to be so much better.

Also, however, it is quite clear that with nutritional knowledge and attitudes as they are, the families whose food expenditures are on the higher economic levels have (on the average) nutritionally better dietaries.

Hence the oft-recurring question whether better nutrition for the people as a whole "is an economic or an educational problem" must certainly be answered that it is *both*.

The majority will undoubtedly provide themselves better dietaries if they have more money to spend for food, even without more knowledge of food values. Also, even without more money to spend for food a better acquaintance with the newer knowledge of nutrition does result in wiser food selection and improved nutritional well-being.

In this country even the lower income groups have some choice in their expenditures, and as rapidly as they come to realize the importance to their children's lives of a greater prominence of the protective foods in the family dietary, they will make the necessary shifts in the family budget. That this degree of understanding of the importance of these foods is not yet sufficiently diffused, and that these foods are still regarded too much as if they were luxuries, is shown by the examples given in Table 56 of differences between dietary practices of families at different economic levels.

TABLE 56. — PER CAPITA CONSUMPTION (IN POUNDS PER YEAR) OF CERTAIN "PROTECTIVE" FOODS IN WHITE FAMILIES SPENDING DIFFERENT AMOUNTS FOR FOOD IN CITIES OF THE NORTH ATLANTIC REGION, AND OF FRUITS AND VEGETABLES IN PACIFIC COAST CITIES

| WEEKLY<br>PER CAPITA<br>EXPENDITURE<br>FOR FOOD | TOTAL<br>FLUID<br>MILK<br>EQUIVA-<br>LENT | CITRUS<br>FRUITS | TOMA-<br>TOES | LEAFY,<br>GREEN AND<br>YELLOW<br>VEGE-<br>TABLES | TOTAL<br>FRUITS<br>AND<br>VEGE-<br>TABLES | EGGS        | FRUITS<br>AND VEGE-<br>TABLES<br>PACIFIC<br>CITIES |
|---|---|------------------|---------------|--|---|-------------|--|
|   | <i>Quarts</i>                             | <i>Lbs.</i>      | <i>Lbs.</i>   | <i>Lbs.</i>                                      | <i>Lbs.</i>                               | <i>Doz.</i> | <i>Lbs.</i>  |
| \$1.25-\$1.87                                   | 123                                       | 16.5             | 19.0          | 46.8   | 170                                       | 13.3        | 360  |
| 1.88- 2.49                                      | 146                                       | 33.8             | 25.4          | 60.1   | 230                                       | 18.6        | 380  |
| 2.50- 3.12                                      | 176                                       | 51.1             | 33.9          | 82.9   | 320                                       | 23.2        | 420  |
| 3.13- 3.74                                      | 181                                       | 66.4             | 37.8          | 92.0   | 400                                       | 27.9        | 550  |
| 3.75- 4.37                                      | 212                                       | 90.4             | 38.4          | 130.1  | 500                                       | 35.5        | 650  |
| 4.38 and<br>over                                | 220                                       | 89.1             | 36.4          | 118.0  | 500                                       | 42.0        | 800  |

From Table 56 it may also be seen that, if the families studied are truly representative, the people of the North Atlantic cities are (or in 1933-37 were) much behind those of the Pacific cities in their appreciation of fruits and vegetables as indicated by consumption at given economic levels.

When the dietaries of families of the North Atlantic region were assessed individually it was found that up to a level of expenditure of \$1.50 a person a week for food all were nutritionally "poor"; and up to \$2.00 none was better than "fair"; but that at every level of food expenditure from about \$2.00 to \$4.50 a person a week, some of the dietaries were "good," some "fair," and some "poor," even when the costs and the markets were the same.

What level of expenditure will support an adequate diet thus depends to a very large extent upon the purchaser's knowledge of food values and appreciation of the importance of nutrition to health, efficiency, and well-being.

### The "Protective" Foods

The term *protective foods* was coined by McCollum and first applied only to milk and the green leaf vegetables, as being the two types of food rich in both the two factors, calcium and vitamin A value, which he had come to regard as most often deficient in the dietaries of American and European peoples.

The research findings of recent years have extended this concept in two ways. We have come to realize that enrichment of



the dietary in vitamin C and riboflavin, as well as in vitamin A and calcium, is usually beneficial; and this is not merely for protection against actual deficiency, but also for the promotion or enhancement of vitality — of “positive,” or “buoyant,” or better-than-average, health. Thus the idea has, perhaps, already outgrown the literal meaning of the word “protective”; but the term continues to do service with this enlarged and more constructive significance.

In this sense, and with the objective broadened to include the enrichment of the diet in the four factors, — calcium, ascorbic acid, riboflavin, and vitamin A, — we now apply the term protective foods to milk, fruit, vegetables, and eggs.\* Milk furnishes all four of the chemical factors just mentioned and is an outstanding source of three of them; while each of the other three types of food here called protective is regarded as a good or excellent source of some two of the four factors. The richness of eggs in vitamin A and riboflavin fully entitles them to a place in the category according to this latter criterion, and the vitamin D and iron content of eggs and the high nutritive value of their proteins are all nutritional assets, especially for the growing child; but eggs do not, like the other protective foods, have the property of reducing intestinal putrefaction and promoting the development of a wholesome bacterial flora in the digestive tract, and for this and some other reasons eggs are more cautiously emphasized, while of milk, fruit, and vegetables we now believe that (within reason) the larger the proportion of the needed calories taken in these three forms the better.

### ***Adequate versus Optimal Nutrition***

The significance of the relation of food to health becomes fully apparent only as feeding experiments upon laboratory animals are followed throughout the entire natural lives of the individuals under observation and through successive generations of families kept upon different diets but under identical living conditions in all other respects.

By means of such experiments it is possible to demonstrate and measure differences of degree in the positive attributes of health such as are included, for example, in the following definition taken from the Century Dictionary: “Health — Soundness of body;

\* Wider definitions of the term “protective foods” have also been proposed.

that condition of a living organism and of its various parts and functions which conduces to efficient and prolonged life; a normal bodily condition. Health implies also, physiologically, the ability to produce offspring fitted to live long and to perform efficiently the ordinary functions of their species."

This is both a positive and comprehensive conception of health, and it is of much interest to note that all of its phases have been covered in studies of the influence of foods upon the health of experimental animals. In human experience so many factors may enter to influence health in the course of a lifetime that it is hard to separate and measure the effects of food alone upon the whole duration and efficiency of life. But this can be, and has been, done with laboratory animals of rapid growth and early maturity, such as the rat, and it has been possible to determine under conditions uniform in all other respects, the influence of modifications of the diet upon the various factors of health comprised in the broad definition above quoted. Among the recent findings of nutrition experiments, carried through successive generations of such laboratory animals, is the fact that starting with a dietary already adequate according to current standards, we may, by enrichment of the diet in certain of its chemical factors through increase in the proportion of protective food, induce a higher degree of health and vigor, and a longer adult life-expectation.

In the writer's laboratory certain families of experimental rats are still thriving in the 50th generation on a diet of which dried whole milk constitutes one sixth and ground whole wheat five sixths of the food mixture. Although this diet (Diet A) is thus unquestionably adequate, an increase in the proportion of milk, from one sixth to one third of the solids of the food mixture (Diet B), results in a marked improvement in nutritional well-being and positive health. Growth is more rapid and more efficient, death-rates are lower and evidences of vitality higher at all ages; in addition to the increase in the average length of life by the diminution of early deaths, there is also a well marked increase in the average length of adult life; and the period of the prime of life is extended in greater ratio than the life cycle itself.

Each of these advances in health through improvement of nutrition has been measured on large numbers of cases in which inheritance and all the factors of the environment except the food were exactly the same.

Special interest has attached to the influence of this improvement of food supply upon the adult life-expectation. This is partly because in the great advance made during the past two or three generations in the life-expectation at birth there had been practically no advance in the life-expectation of the adult. The diminution of death rates had been practically confined to the early ages. And, moreover, studies on longevity had succeeded only in correlating it with heredity. Hence the present experimental correlation of length of adult life with an improvement in an already adequate food supply was a finding unexpected to those to whom we had previously owed our chief knowledge in this field; and it is optimistic and constructive where the previous view had been pessimistic and fatalistic.

Another reason for special interest in these findings is that here the *same* improvement, induced by a more scientific quantitative proportioning of everyday articles of food, has *both* expedited growth and development *and* advanced the vitality and life-expectation of the adult; so that longer life here means not a longer old age but a longer "period of the prime," and the life "cycle" or "course" is not only longer but is lived on a higher level of positive or buoyant health throughout.

That the increase in the adult life-expectation is of convincing magnitude is shown by the data of Table 57 where the difference for males is 5.6 and for females is 5.9 times the respective probable errors.

TABLE 57. — COMPARISON OF LENGTH OF LIFE OF RATS ON DIETS A AND B

|             | ON DIET A          |                                   | ON DIET B          |                                   | DIFFERENCE<br>OF LENGTH<br>OF LIFE<br>IN DAYS |
|-------------|--------------------|-----------------------------------|--------------------|-----------------------------------|---|
|             | Number<br>of Cases | Average Length<br>of Life in Days | Number<br>of Cases | Average Length<br>of Life in Days |   |
| Males . . . | 135                | 571 $\pm$ 8.0                     | 124                | 635 $\pm$ 8.5                     | 64 $\pm$ 11.7                                 |
| Females .   | 196                | 603 $\pm$ 8.0                     | 163                | 669 $\pm$ 7.8                     | 66 $\pm$ 11.2                                 |

And the data become still more convincing and significant when arranged as in Table 58 from which it may be seen that the prospect of reaching a given age was, at every one of nine points of comparison, distinctly higher upon Diet B than upon Diet A.

It is also of interest, and of far-reaching significance in its implications for the improbability of man, that even those families

TABLE 58. — INFLUENCE OF FOOD UPON ATTAINMENT OF DEFINITE AGES

| PERCENTAGE OF<br>LIVES LONGER<br>THAN: | MALES     |           | FEMALES   |           |
|--|-----------|-----------|-----------|-----------|
|  | On Diet A | On Diet B | On Diet A | On Diet B |
| 600 days . . .                         | 42.9      | 65.3      | 54.1      | 73.0      |
| 700 days . . .                         | 14.8      | 32.3      | 27.6      | 43.6      |
| 800 days . . .                         | 2.9       | 10.5      | 12.2      | 15.9      |
| 900 days . . .                         | 0.0       | 1.6       | 2.6       | 5.5       |
| 1000 days . . .                        | 0.0       | 0.0       | 0.5       | 1.2       |

which had been habituated to the "minimum-adequate" Diet A through many generations were still able to respond in the same manner as the more fortunate families to the improvement in food supply represented by the transfer from Diet A to Diet B (Sherman and Campbell, 1936).

The better of these two diets is probably capable of further improvement.

Here there may perhaps be a need to safeguard against either of opposite misinterpretations. On the one hand, the over-enthusiastic or the sensational may exaggerate the scientific finding or its human implications, and extrapolate the discussion into extravagant suggestions. On the other hand, the over-cautious or the minimizer may tend to interpret as merely the correction of a deficiency what is really a constructive advance.

The superior health, which often results from improvement in an already adequate diet, is a condition within the range of the normal as already known; but the marked improvement of the average is probably of far-reaching significance.

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## CHAPTER XXIX

### FOOD ECONOMICS IN THE LIGHT OF THE NEWER CHEMISTRY OF NUTRITION

#### Nutritional Research Helps to Reform "the Backward Art of Spending Money"

AT LEAST one school of economic thought has been criticized on the ground that it "knows the price but not the value." A distinguished economist of another school, thinking more in terms of values, wrote an essay (which was so well regarded as to be republished at least a decade later) on "The Backward Art of Spending Money." Its argument was that the 'exact sciences' of physics and chemistry guide efficiently and progressively the productive industries by which goods, and through which incomes, are made; while the spending of the income in the hope of obtaining thereby a satisfying life remains a backward art because it has the guidance of only such inexact sciences as psychology and sociology.

In the few years since that essay was reprinted, chemistry has accomplished more than could have been foreseen to guide the use of that most outstanding of all material resources, the food supply, in such manner as to make healthier and happier lives, more satisfying and of greater social value.

The spending of money for food, and the judgment of the proper place of food in the total budget of the individual or the family, have both been greatly enlightened by recent chemical investigations of nutritive values and of the relations of nutrition to health and to the extension of the prime of life.

As the Federal Department of Agriculture has officially recognized, economic conditions and the growth of scientific knowledge have focused attention upon the problems of food budgets. "The consumer, interested in getting good returns for what he can afford to spend, wishes to have this information interpreted at several economic levels. The producer . . . wants to know how much of different foods may well appear in the diets of different

consumer groups and to what extent consumption may rise or fall as the economic situation changes."

Undoubtedly a large proportion of our people still consume much less of the protective foods than would be best. Milo Perkins states, in Harper's Magazine of December 1940, that two thirds of the people of the United States live on an average cash income of only \$69 a month for a whole family, and that this means "a shocking amount of under-consumption." He estimates that, "If all the families making less than \$100 a month ate as much as those which do make \$100 a month, nearly two billion dollars a year would be added to the national food bill." And instead of there being so-called food surpluses, the home market would justify some increases over present production in order to feed all our lower-income people according to the standard of living of \$1200 per family per year, those of higher income continuing their present food practice.

At a level of twelve hundred dollars per family per year, a little over 30 per cent of the income would have to be spent for food to permit the investment of one dollar a day in the purchase of the entire food of the family. This is only 20 cents per person per day in a family of five, or 25 cents in a family of four. Or, one fourth more could be spent for food if other expenditures were reduced one tenth.

As explained in previous chapters and in the one which follows, recent advances in our knowledge show that nutrition plays a much greater part in welfare and in the economic and social values of one's work than hitherto supposed.

While our newest nutritional knowledge affords fuller and clearer guidance for the problem of the best investment of money in food, it can hardly be said that the problem has been made easier. The opportunity is stimulating but the responsibility is heavy. Under the best economic conditions which we can foresee for the near future, it will be no easy matter for every home-maker to feed her family in such a way as to give fullest nutritional support to the innate potentialities of each individual. It seems appropriate that our present study include a consideration of the principles which should serve the present-day efforts to bring nutritional knowledge as fully and promptly as possible into the service of food economics and of public health and welfare. Here there is a three-fold need and opportunity:



(1) Openminded and thoroughgoing study of the individual or family problem as to how the money which is *being* spent for food may now best be invested. There is need to emphasize the fact that this is a question to which fundamentally important new evidence has been brought by very recent research. So far as space permits, the new evidence has been mentioned in previous chapters, and in this and the following chapter we are considering how this newer knowledge should function in our scientific attitude toward the everyday choice and use of food, and what may be expected of a more scientifically guided food habit. Certainly a large majority of individuals and families may expect, in the course of a life-time, very important benefits from the forming of such daily food habits as make use of this newest knowledge of nutrition.

(2) Because the potentialities of nutrition for the improvement of the life process and for the individual and family satisfaction in life are now found to be greater than expected, there is need for reappraisal of the *relation between expenditures for food and for other things*. The rather prevalent attitude of dismissing this problem on the ground that "the American standard of living demands" such-and-such expenditures in other directions becomes no longer valid when one grasps the fact that a moderate diversion from expenditure in some other direction for the sake of increased investment in food under the guidance of the newer knowledge of nutrition has a very high potentiality for bringing increased satisfaction in life.

(3) The same nutritional improvement of the life process, which may mean so much to oneself, also increases the economic and social value of the individual to the community and so builds a stronger nation of abler, happier, and more useful citizens. Hence the public health and general welfare of the nation demand that we extend to all the people the newer and higher standards of nutritional wellbeing. True as it is that the dissemination of modern knowledge of nutrition and food values, even in very simplified form, will enable most home-makers to improve the feeding of their families even on their present incomes; yet it is also true that a disturbingly large proportion of families have incomes so small that they could hardly be expected to be so fed as *fully* to develop their innate potentialities, however well instructed and conscientious in the choice and use of food. For the

nation as a whole to protect its own economic and health interests, there must be effective recognition of the fact that the problem is both educational and economic. *Better levels of consumption* of the right kinds of food should be taught to all the people; and, in addition, *should be made possible* for those of low income either by in some way increasing their purchasing power or by subsidized distribution of the actual articles of food whose increased consumption is most directly indicated by nutritional knowledge. If, for instance, the "food stamp plan" is continued, it might well be directed primarily to present-day nutritional objectives. There is now ample scientific basis for confidence that the cost of both the educational work and the needed economic measures will within a generation be repaid many fold in the higher health and improved working efficiency of the people.

Each of the above three aspects of the general problem presents difficulties. People are apt to be slow to change their established habits, particularly in such personal matters as their daily food; there is reluctance to increase governmental paternalism; and there also tends to be a sort of "vested interest" attitude of resentment toward any teaching of readjustment of consumers' expenditures.

For these and other reasons, suggestions that we change our food habits into better accord with the new knowledge of nutrition have been made only very cautiously. As we read such recommendations or suggestions in this chapter or in other books or bulletins, it will be soundly scientific to ask ourselves whether we might derive fuller benefit from the new knowledge if we went at least a little farther in giving increased prominence to fruits, vegetables, and milk in our dietaries than do the recommendations that we meet in print.

Such fuller use of our scientific knowledge need not involve giving up anything.

We can use the guidance of the newer knowledge of nutrition and continue to use every kind of food. As McCollum teaches: Eat what one should, and one may also eat what one likes.

The development and diffusion of nutritional knowledge is already affecting the relative prominence of some articles of food in the typical consumer's budget, and such adaptation of emphases in food habit to our growing nutritional knowledge will probably go farther; but every species and variety of food we now

know will doubtless continue to be produced, to find a market, and to be as readily available to consumers as it now is. If a shift of emphasis in consumer demand should make it profitable for the mid-western farmer to convert a larger proportion of his corn and alfalfa into milk and eggs, he will still be producing meat also; and a little farther west are great areas of grazing lands which will continue to send their present large output of meat to our markets.

### Grouping of Foods for Dietary Planning

Stiebeling and coworkers have developed a plan of *twelve food groups* instead of the five to ten of the discussions in Chapter XXVIII. Obviously, the larger the number of groups the more nearly interchangeable the members of each group can be. These twelve groups and their use in planning dietaries are discussed on pages 35-36 and 323-340 of "Food and Life," the 1939 Yearbook of the United States Department of Agriculture, and in Chapter XX and Appendix F of Sherman and Lanford's *Essentials of Nutrition*.

The twelve groups as summarized in the Yearbook just mentioned are: (1) milk; (2) potatoes and sweetpotatoes; (3) dry mature beans, peas, and nuts; (4) tomatoes and citrus fruits; (5) leafy, green, and yellow vegetables; (6) other vegetables and fruits; (7) eggs; (8) lean meat, poultry, and fish; (9) flours and cereals; (10) butter; (11) other fats; (12) sugars.

This grouping of all kinds of foods into twelve categories suggests the following comments from the nutritional point of view: (a) With milk, whether fresh or fermented, would be included the fluid milk equivalents of any canned and dried milks used, and also cheese, cream, and ice cream as foods which retain the essential nutritional characteristics of milk. (b) While potatoes and sweetpotatoes are largely interchangeable in the food supply, sweetpotatoes are of much the higher vitamin A value. (c) Lentils and any other mature legumes obviously would be assigned to the same group with dry beans, peas, and nuts. (d) Canned tomatoes, canned grapefruit, and canned grapefruit juice have been definitely shown by investigations to retain the original vitamin values of these foods in exceptionally high degree. (e) In some respects but not in all, there are marked differences between the green outer leaves and the whitish inner leaves of headed cabbage and lettuce; and correspondingly between the looseleaf and the

TABLE 59. — AVERAGE DISTRIBUTION OF EXPENDITURES AMONG SPECIFIED GROUPS OF FOODS, AND PROPORTION OF NUTRITIVE CONTENT CONTRIBUTED BY EACH FOOD GROUP

(26 East North Central families spending \$1.88-\$2.49 weekly per capita for food, spring 1935)

(Stiebeling and Phipard, 1939)

| Food Group                                       | PROPORTION<br>of MONEY<br>SPENT FOR— | PROPORTION FURNISHED OF— |          |          |                 |          |                  |          |                 |                         |
|--|--------------------------------------|--------------------------|----------|----------|-----------------|----------|------------------|----------|-----------------|-------------------------|
|  |                                      | Calories                 | Protein  | Calcium  | Phos-<br>phorus | Iron     | Ascorbic<br>Acid | Thiamin  | Ribo-<br>flavin | Vita-<br>min A<br>Value |
|  | Per cent                             | Per cent                 | Per cent | Per cent | Per cent        | Per cent | Per cent         | Per cent | Per cent        | Per cent                |
| Eggs . . . . .                                   | 5.4                                  | 2.5                      | 7.5      | 4.2      | 6.3             | 10.0     | 0                | 4.5      | 8.0             | 8.0                     |
| Milk, cheese, ice cream . .                      | 12.1                                 | 10.0                     | 17.4     | 64.0     | 28.5            | 6.6      | 5.3              | 13.6     | 35.4            | 13.1                    |
| Butter, cream . . . . .                          | 4.9                                  | 6.0                      | .3       | .9       | .5              | .4       | 0                | .1       | .2              | 9.3                     |
| Other fats, including fat meat                   | 5.6                                  | 12.7                     | 1.3      | .4       | .8              | .9       | 0                | 2.6      | .7              | *                       |
| Lean meat, poultry, fish . .                     | 24.2                                 | 11.6                     | 31.8     | 2.2      | 21.8            | 28.5     | .8               | 31.4     | 28.6            | 7.5                     |
| Sugars . . . . .                                 | 3.4                                  | 10.7                     | .1       | .8       | .2              | 1.0      | .7               | *        | 0               | *                       |
| Bread, other baked goods . .                     | 13.6                                 | 19.8                     | 16.2     | 8.4      | 11.0            | 10.7     | 1.3              | 4.3      | 5.0             | 3.8                     |
| Ready-to-eat cereals . . . .                     | .9                                   | .8                       | .8       | .3       | 1.6             | 1.3      | 0                | 1.1      | .4              | *                       |
| Flour, other cereals . . . .                     | 3.6                                  | 10.7                     | 11.2     | 2.5      | 7.6             | 7.2      | .1               | 2.3      | .1              | .3                      |
| Potatoes, sweetpotatoes . .                      | 2.2                                  | 6.1                      | 4.6      | 3.7      | 8.3             | 12.5     | 23.7             | 13.4     | 7.7             | 11.2                    |
| Mature beans and peas, nuts                      | .9                                   | 1.6                      | 3.0      | 1.9      | 3.7             | 5.2      | 0                | 6.6      | .6              | 0                       |
| Leafy, green, and yellow<br>vegetables . . . . . | 5.1                                  | 1.1                      | 2.1      | 3.5      | 3.1             | 5.4      | 15.0             | 5.0      | 4.3             | 32.8                    |
| Tomatoes . . . . .                               | 1.7                                  | .3                       | .4       | .5       | .6              | .8       | 7.8              | 2.1      | .6              | 8.4                     |
| Other vegetables . . . . .                       | 1.8                                  | .7                       | .8       | 1.6      | 1.3             | 1.2      | 5.0              | 1.5      | 1.0             | .4                      |
| Citrus fruits . . . . .                          | 2.2                                  | .7                       | .3       | 1.9      | .6              | 1.2      | 19.1             | 3.4      | 2.1             | .3                      |
| Other fruits . . . . .                           | 6.0                                  | 3.6                      | 1.0      | 2.6      | 2.4             | 6.1      | 21.0             | 5.5      | 4.4             | 4.6                     |
| Miscellaneous . . . . .                          | 6.4                                  | 1.1                      | 1.2      | .6       | 1.7             | 1.0      | .2               | .6       | .9              | .3                      |
| Total . . . . .                                  | 100.0                                | 100.0                    | 100.0    | 100.0    | 100.0           | 100.0    | 100.0            | 100.0    | 100.0           | 100.0                   |

\* Less than 0.1 per cent.

headed varieties. (f) Breadstuffs will naturally be counted with flour and cereals; other bakery products, probably here also.

The contributions of different nutrients, compared with the cost, of each type of food in percentage of the total food supply may be studied in the accompanying Table 59.

Table 59 from the work of Stiebeling and Phipard shows a presumably typical distribution of expenditure among different articles and types of food and the contribution of each of these toward the total of each of nine nutritional factors: Calories, protein, calcium, phosphorus, iron, ascorbic acid, thiamin, riboflavin, and vitamin A value. In the light of present knowledge of the health significance of each of these factors, does the distribution of food money among the different food groups represented in Table 59 admit of improvement? To what extent would the answer depend upon local and seasonal conditions of food supply?

Using data from the tables in the Appendix, readers may similarly compute what they obtain in nutritive values from their own expenditures for foods. This is the most important food question for the consumer. Too much of the advice currently addressed to consumers is written as if the consumer's problem were chiefly one of avoiding being deceived by the seller, whereas in fact a much more important consumer problem is that of deciding what things to buy and how much of each. Not only is the latter the more important, it is also the more permanent, consumer problem. For the food laws with their National, State, and local inspection services are, together with Federal trade regulation, rapidly relieving the consumer of the police function of preventing fraud; while the decision upon how to invest the food money remains a function of the consumer, and is steadily growing a more and more weighty responsibility as research reveals the unexpectedly great and far-reaching influence of nutrition upon health, efficiency, earning power, satisfaction in life, and social value to one's community and times. Thus the fact bears repetition that the best thought of the consumer should be devoted to the making of full use of scientific guidance in deciding what foods to buy and in what relative proportions to buy them.

### Six Plans for Different Circumstances

The four dietary plans suggested, for different economic levels, by Stiebeling and Ward in 1933 were designated as: "(1) Re-

stricted diet for emergency use; (2) Adequate diet at minimum cost; (3) Adequate diet at moderate cost; (4) Liberal diet." The subsequently suggested "Diet Plans A and B" (Stiebelling, 1935) offer modifications which from the economic viewpoint are adapted to conditions which permit of the home-growing of significant parts of the food supply, and which also reflect the guidance of the newer knowledge of nutrition even more fully perhaps than do the suggestions of 1933.

Omitting from the discussion here the lowest-cost or "restricted" diet as having been suggested only for emergency use, Table 60 summarizes the Plans 2, 3, and 4 of 1933, and the plans A and B of 1935; and adds 3A and 3B which combine the features of the plan 3 of 1933 with the plans A and B of 1935. The figures given are for per capita consumption yearly of the chief types of food as grouped in the light of present-day knowledge of nutritive values.

TABLE 60. — YEARLY PER CAPITA FOOD CONSUMPTION ACCORDING TO DIFFERENT PLANS

| KIND OF FOOD  | PLAN<br>2 | PLAN<br>3 | PLAN<br>4 | PLAN<br>A | PLAN<br>B | PLAN<br>3A | PLAN<br>3B |
|---|-----------|-----------|-----------|-----------|-----------|------------|------------|
| Flour, cereals . . . . . pounds                               | 224       | 160       | 100       | 195       | 175       | 160        | 160        |
| Milk (or equivalent)* . . . quarts                            | 260       | 305       | 305       | 260       | 260       | 305*       | 305        |
| Potatoes, sweetpotatoes . . pounds                            | 165       | 165       | 155       | 155       | 145       | 165        | 165        |
| Dried beans, peas, nuts . . pounds                            | 30        | 20        | 7         | 25        | 15        | 20         | 20         |
| Tomatoes, citrus fruits . . pounds                            | 50        | 90        | 110       | 105       | 85        | 105        | 105        |
| Leafy, green, and yellow<br>vegetables . . . . . pounds       | 80        | 100       | 135       | 130       | 100       | 130        | 100        |
| Dried fruits . . . . . pounds                                 | 20        | 25        | 20        | ?         | ?         | ?          | ?          |
| Other vegetables, fruits . . pounds                           | 85        | 210       | 325       | 400       | 330       | 400        | 330        |
| Fats (including butter,<br>oils, bacon, salt pork) . . pounds | 49        | 52        | 52        | 45        | 50        | 50         | 50         |
| Sugars . . . . . pounds                                       | 35        | 60        | 60        | 50        | 50        | 50         | 50         |
| Lean meat, poultry, fish . . pounds                           | 60        | 100       | 165       | 25        | 55        | 25         | 55         |
| Eggs . . . . . dozen  | 15        | 15        | 30        | 10        | 20        | 15         | 20         |

\* The approximate equivalents are, as given by the U. S. Dept. Agriculture, for 1 quart of fluid whole milk: 17 ounces of evaporated milk; 1 quart of fluid skim milk and 1.5 ounces of butter; 5 ounces of American Cheddar cheese; 4.5 ounces of dried whole milk; 3.5 ounces of dried skim milk and 1.5 ounces of butter.

As we saw in the preceding chapter, and as explained more fully in the writer's *Food Products*, American families differ widely in their food budgets and per capita consumption of the different articles and types of food, even when living on the same economic level.

When the newer chemistry of nutrition suggests a greater prominence of fruits, of vegetables, and of milk, cheese, cream, or ice cream in the American dietary, it is not suggesting anything unprecedented. Large numbers of American families have furnished the precedent already and have found satisfaction in this more modern plan, and in the superior health and efficiency which it induces. A gradual trend of the national food consumption, in the direction indicated as beneficial by our newer nutritional knowledge, means that the example of those who are already benefiting by this knowledge is being followed by one after another of their neighbors.

And the trend to greater prominence of fruits, vegetables, and dairy products in the family dietary and national food economy is progress for agriculture also. As expressed in an official publication of the United States Department of Agriculture, the general use of such dietaries "would not only improve the health and efficiency of the population, but at the same time would foster the type of agriculture which represents wise utilization of land for the country as a whole."

Stiebeling and Ward's (1933) estimates of foods needed per capita to meet American consumer demand at different levels of purchasing power are based in part upon the relations which have been observed in the past between purchasing powers and consumers' choices in the United States, and in part upon their judgment of what must be consumed if the food supply is to be regarded as fully satisfactory nutritionally in the light of the knowledge of 1931-33. The still more recent findings of the chemistry of nutrition indicate that we may well go farther in the same direction of progress which their recommendations were designed to promote.

Partly but not entirely because of the recent evidence that the optimal calcium intake is significantly higher than is provided by hitherto approved dietary standards, increasing numbers of people guided by this newer chemistry of nutrition will doubtless follow the advice frequently given by McCollum and include a quart of milk per day in their customary food consumption.

Together with increased milk consumption there will probably continue to go an increased consumption of fruits and vegetables, especially those hitherto regarded as luxuries and now seen to be good dietary investments.

Because fruits and vegetables differ so much among themselves

and in the ways that they are served, it is harder here than in the case of milk to compare the consumption levels of different individuals or families in as accurate a way. Yet there is no room for doubt that with growing acceptance of the guidance which our newer knowledge offers, fruits and vegetables are being given an ever greater prominence in the dietary.

Thus one student of nutrition consumed in the course of a recent year: 1577 servings of fruit and fruit products; 275 servings of tomato or tomato juice; 1407 servings of other vegetables. During the same year the total consumption of meats, poultry, fish, and shellfish was 300 servings; of eggs, 116 servings. The servings of fruits, vegetables, and their products were of at least average conventional sizes; so that probably the 3259 servings or portions consumed in the year represented at least 800 pounds of fruits and vegetables as they come to the kitchen, or the same as the highest levels of fruit and vegetable consumption recommended by the U. S. Department of Agriculture in 1933 or in 1935. The servings of meat averaged less than conventional size: probably meat consumption here was near the level of the lowest figures which the Department of Agriculture has recommended (Plans 2, A, and B of Table 60). The 116 servings of eggs in the same year were about as often of one as of two so that the consumption was probably about 12 to 15 dozen, or of the same order as all but the most liberal of the plans shown in Table 60. Here a food habit guided by the newer knowledge of nutrition and giving more than average prominence to fruits, vegetables, and milk, at the same time afforded greater pleasure than the more conventional American food habit of the past, and at somewhat less cost.

Later, the same student of nutrition found that the place of fruit in his dietary had progressed to a higher level. In a year in which they were abundant, he consumed 868 pounds of citrus fruit as well as usual amounts of other fruits.

Most families and most individuals undoubtedly could build health to higher levels by giving to fruit and to milk in its various forms a larger place in the dietary. This is possible, even with present purchasing power, by moderate readjustment of expenditure: partly by shifts within the food budget; partly by economizing in other directions in order to have more to spend for food. Cost-of-living studies show that even among people of the lower income groups some money could be shifted to the purchase of



more fruit and of more of whatever form of milk, cheese, cream, or ice cream one likes best, from present expenditures for adornment-other-than-clothing and amusement-other-than-automobile. Once the significance of the gains is understood, this does not mean deprivation but rather increase of satisfaction. Fruits and dairy products can be so selected and used as to add much to the pleasures of the table. And the satisfaction of improving the buoyancy of health, the efficiency, and the level of achievement of all its members is the highest and most lasting pleasure that a family can derive from its income.

### **Dietary Adjustments and the National Food Supply**

As has been briefly noted earlier in this chapter, the general adoption of a dietary such as we now believe to be best would undoubtedly help the natural evolution of American agriculture. For a given amount of materials consumed, a dairy herd yields a product of much greater food value than does a herd of beef animals. Armsby estimated that of the energy value of what the animal consumes, about 18 per cent is recovered for human consumption in milk and less than 4 per cent in beef. In the conservation of calcium and of vitamin A values the difference in favor of the dairy animal is still greater. With the development of a more intensive agriculture the inherently greater economy of dairy cattle over cattle raised merely for slaughter naturally tends toward a shifting in the proportions of the two types. Regions adapted to dairy farming but too remote from large markets to ship milk in the fresh state, can now market their dairy production as canned and dried milk; and also, of course, as butter and cheese. Yet because of its great areas of range pastures America will continue to have an abundant meat supply.

The acreage of fruit and vegetables is so readily expanded that production can easily keep pace with a largely increased consumer demand, though the concentration of population in large cities increases the expense of transportation and makes the cost of retail distribution a serious item, especially in the case of bulky products with a relatively low money value per pound, such as cabbage, potatoes, and root crops. The more highly perishable fruits and vegetables having a higher cost per pound or ton are now successfully transported in transcontinental carload shipments. Pre-cooling and lowered temperatures in refrigerator cars

promise to reduce still further the losses incident to their transportation. And meanwhile we have learned through research in the chemistry of foods and nutrition that many of these perishable fruits and fresh vegetables, formerly regarded as luxuries, are in fact good investments for a liberal share of the consumer's food money, because of their richness in nutritional factors in which it is desirable that our dietaries be further enriched.

In order that a nutritionally guided consumer demand shall increase the supply of the desired foods without materially increasing prices, it is important that the science of nutrition offer its practical advice to the art of dietetics in terms of foods that are crops-in-themselves, not merely by-products. Liver has therapeutic value in pernicious anemia; but Rose finds that for normal nutrition, including the requirements of rapidly growing children, egg does all that liver can do. Obviously, then, if one of these is to be emphasized in the teaching of nutrition and food values to the public it should be the egg. For eggs are a crop in themselves, whereas the supply of liver can only be increased by rearing and slaughtering animals in which liver is but a small by-product.

### **Significance of Home Gardens for Nutrition and Food Economics**

In the 1940 symposium, "Nutrition for the Higher Health," given by the American Institute of Nutrition, McCollum, speaking on the problem of better nutrition for the people, gave the major part of his time and emphasis to the desirability of increasing home gardening, pointing out its many other values as well as its contribution to the family food supply.

Only the latter aspect can be considered here. The frequent tendency to dismiss the subject of home-grown food as economically unimportant is beside the point. Doubtless it is true that what is grown in home gardens will have no appreciable effect upon any important food industry. Recognition of this should reassure the agricultural economist and prevent undue expectations in the amateur gardener, or even the part-time farmer whose home gardening approaches the ambitions of a subsistence homestead.

The agricultural economist need not fear, and the home gardener will be wiser not to hope, that the products of home gardens will materially influence the food markets.

Yet the home gardens can be of very real and significant benefit through the fact that each family with a garden can grow an *extra*

supply of fruits and vegetables to be consumed *in addition* to all that they have been accustomed and able to buy. This can mean very material improvement of nutritional well-being and resultant health, happiness, and efficiency; and the means for it can be found by a constantly increasing proportion of people as they come to realize how great a benefit it can be to themselves and (still more) to their children.

The real point here, which could not be clearly realized before we had the light of the newer chemistry of nutrition, is that, when the demand of the market has been fully met, in the agricultural-economic sense that consumers have bought all they can buy at a price at which producers and distributors can afford to sell, the average consumer family still does not have as much of fruit and fresh vegetables as it needs if it is to realize its full potentiality of health.

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## CHAPTER XXX

### NUTRITIONAL CHEMISTRY AND HUMAN PROGRESS

#### Scientific Recognition of the Far-Reaching Significance of Nutrition

McCOLLUM has taught for at least twenty years that there are often important differences between the merely adequate and the optimal in nutrition; and simultaneously, J. F. Williams, doctor of medicine and professor of health education, has taught that health is not merely the absence of disease, but a positive quality of life which can be built to higher levels.

After a decade of such teaching Williams still remarked upon the slowness of general acceptance of this view. During the past decade, however, the constructive possibilities of nutrition have received increased recognition.

In 1931, British scientists established the international quarterly *Nutrition Abstracts and Reviews*, published by the Aberdeen University Press, with editors in thirty countries. Its first review was written by Sir Frederick Gowland Hopkins, then President of the Royal Society as well as professor of biochemistry in Cambridge University, and was entitled, "Nutrition and Human Welfare." Hopkins pointed out that the importance of nutrition had doubtless often been missed in studies not conducted under conditions of strict scientific control, and that a supposed racial inferiority may be only the effect of an unrecognized inferiority of the food supply. He considered that the true evidence would show that few races have at any time been ideally nourished; that the kinds and relative amounts of food available "have played a great part in determining the destiny of races"; and that the strictly controlled scientific research which was becoming established at that time (1931) "wholly changed the outlook," calling for an abandonment, in the domain of nutrition, of tradition (which, he said, "accumulates prejudices quite as often as truths") and the use of the new knowledge, gained through controlled experiments, for the advancement of human welfare.

In 1932 Sir Walter Fletcher, English physician and member of the British Medical Research Council, wrote that whereas in the physical sciences fundamental advances of knowledge usually undergo some decades of theoretical development before invention brings them into the service of daily life, with the new science of nutrition discovery and application so stimulate each other that: "A flood of new results is pouring every day from the laboratories, the field stations, the hospitals, and the farms, where keen interest has been aroused either by the deep-seated physical and biological relations that are being revealed or by the new powers that are being given of practical control over health in animal and man." Never, he considered, has there been a more complete fusion of theoretical interest and practical significance. He pointed out to physicians and health officers that experimental researches in nutrition "are continually revealing new and unsuspected biological relationships of fundamental kind. . . ." Thus, he emphasized that the experiments of Dr. Corry Mann upon diets for schoolboys (noted in connection with our discussion of growth and development in Chapter XXVI) revealed much more than had been foreseen. For they showed that, starting with a diet which medical opinion adjudged to be sufficient for healthy development, an extra allowance of protective food improved both physical and mental growth.

In 1935 Dr. J. S. McLester, in his official address as President of the American Medical Association, said that science promises to those peoples who will use the newer knowledge of nutrition, greater vigor, increased longevity, and a higher level of cultural attainment.

In 1936 Sir John Boyd Orr, director of the Imperial Bureau and Rowett Research Institute of Nutrition wrote in his *Food, Health, and Income*: "This new knowledge of nutrition, which shows that there can be an enormous improvement in the health and physique of the nation, coming at the same time with the greatly increased powers of producing food, has created an entirely new situation which demands economic statesmanship."

Dr. Frank M. Boudreau, speaking from the vantage point of his experience as director first of the Health Organization of the League of Nations and now of the Milbank Memorial Fund, has recently said that "the benefits of an abundantly adequate diet are greater than we had any reason to expect."

Today's scientific recognition of the unexpectedly great and far-reaching potentialities of the science of nutrition is doubtless due to a combination of causes.

It is a natural fruition of the teachings of such leaders as have been mentioned above and others who would be mentioned if space permitted.

That so many careful scientists now speak with so much confidence is in turn very largely attributable to the further consideration that whereas at first such statements were regarded as matters of opinion, the most recent years have seen such developments of research methods, perfection of experimental control, increased numbers of observations, extension of experiments throughout the entire lives of successive generations, and statistical scrutiny of results, that the findings have rapidly acquired more of the status of objectively established fact.

In some cases the experimental methods have been so developed as to give the findings a previously unattempted degree of comprehensiveness and far-reaching significance as explained in the sections which follow.

### Increasingly Comprehensive Methods of Research

In the researches which have been directed particularly toward the nutritional problems of entire lifetimes and successive generations, the *experimental variables* have been of two kinds: (1) the *individual chemical factor*, element or compound as the case may be; and (2) the *article of food* as produced by nature or agriculture and as actually consumed in daily life.

The food shortages occasioned by the World War of 1914-18 stimulated experimentation in terms of actual articles of food, and by 1930 it had been shown that a dietary made up of everyday food materials may be permanently adequate to the needs of normal nutrition, including those of growth, reproduction, and lactation, generation after generation, and yet that nutritional well-being and length of life may be improved by a more scientific adjustment of the everyday foods in the dietary. One such investigation has been described in the section on adequate *versus* optimal nutrition in Chapter XXVIII. Here the sole experimental variation was in the quantitative proportions in which the natural foods were fed; but when translated from articles of food to the individual chemical factors, the dietary difference involved four

enrichments, — a relatively small increase in the protein content of the food supply and relatively liberal increases in its calcium and riboflavin contents and its vitamin A value.

Subsequent investigation has therefore sought to show which of these were the influential factors and what degrees or levels of enrichment with each is most conducive to nutritional well-being as revealed in experiments extending throughout the entire life cycles of successive generations.

And as it is always to be kept in mind that there may be still-undiscovered substances essential to nutrition, comprehensive experiments were also made to test the effect of diversifying the diet with natural foods of other types.

Both when the diet was thus diversified and when enriched in protein content by addition of casein, there resulted somewhat more rapid growth but no detectable gain in vitality or length of life. Thus protein-enrichment can have had only a minor effect in the above-noted improvement of an already-adequate diet; and it is also rendered improbable that any undiscovered factor was significantly influential in this investigation. Moreover, the separate experiments with calcium, riboflavin, and vitamin A revealed effects which seem adequately to account for the nutritional improvement previously demonstrated in the above-mentioned experiments with natural foods. Those of the findings on these three factors which are chiefly significant from the viewpoint of the present chapter are, therefore, briefly summarized in the following paragraphs.

Calcium was found to account for a large part of the nutritional improvement which had resulted from increasing the proportion of protective food in an already-adequate diet. In an extended series of experiments with diets differing in their calcium contents only to the same extent as in the above-mentioned mixtures of natural food materials (Sherman and Campbell, 1935, 1937) this moderate increase of calcium intake resulted in slightly more rapid growth, more efficient utilization of food value whether computed in terms of calories or of protein, slightly earlier maturity, better success in reproduction and lactation, and some increase in the average length of adult life. Here the gain in longevity by the males was undoubtedly significant while that by the females was less and if it stood alone would not be statistically convincing. But the females receiving more calcium had borne and reared



more young. That they invested their extra calcium in more and better (Chapters XIV and XXVI) offspring and did not inherently lack the ability to profit in the same way as the males, is shown by the results of two subsequent series of experiments, as follows: (1) It was found that the above-described increment of calcium intake did increase the longevity of unmated females. (2) When the increase in the calcium content of the diet was more liberal there resulted both increased success in reproduction and lactation and the attainment of greater longevity by the same individual females.

Such findings as these illustrate the steadily developing possibilities of direct and well controlled laboratory investigation of problems of far-reaching human importance, by studies of the nutritional responses of properly chosen experimental animals throughout their entire life-times, and in some problems through successive generations.

Riboflavin, while functioning so differently in the body, has been found like calcium to confer increasingly beneficial results with increasingly liberal intakes up to levels from two to four times as high as that of minimal adequacy for permanently normal nutrition. And it may prove of much significance for continuing human progress that further increases in the riboflavin content of the diet, beyond the zone in which the individuals of the first generation show immediately tangible response, still seem to confer additional benefit upon the offspring (Chapter XIX). The most probable interpretation of these findings appears to be, that the benefit to the internal environment of the mother's body is more delicately reflected in her offspring than in what we yet have means to measure in her own adult organism. Fuller chemical explanation of the upper ranges of the wide zone between minimal-adequate and optimal intake of riboflavin may be expected from the combined results of (1) studies of the molecular structures of the tissue enzymes and coenzymes and the exact nature of the chemical reactions which they catalyze, and (2) determinations of riboflavin in offspring of differently fed experimental animal families at early ages by improved methods. And fuller understanding of these chemical factors will doubtless permit a clearer appraisal of the human significance of this far-reaching effect of the nutritional improvement of an already-normal life process.

Vitamin A, as we saw in Chapter XXII, was found by Batchelder to yield increasingly beneficial results with increasing liberality of

intake up to about four times the minimal adequate level; and an analogous four-fold zone was found by Mellanby and Green in their studies of the influence of vitamin A (or of carotene) upon ability to survive experimental infections. In an extended series of later experiments belonging to the research upon which the present section chiefly draws for further illustration (Sherman and Campbell, 1937) it has been found that vitamin A was clearly a significant factor, along with calcium and riboflavin, in the previously reported nutritional improvement of an already-adequate diet. The data of these experiments also give interesting indications that extra liberal levels of intake of vitamin A may have an even greater influence in the maintenance of high health and low death rates in the adults than in the young. In human affairs this would mean specifically enhanced opportunity for both the individual and the community to realize more fully upon the investment involved in his education and years of acquirement of skill and experience. It is thus of special interest from the viewpoint of this chapter that further experimental studies of the effects of different levels of vitamin A intake in the building of higher health are in progress as this is written.

While, as explained in Chapters XXVI and XXVII, the zone between the minimal-adequate and optimal levels of intake is not equally wide for all nutrients, and calcium, riboflavin, and vitamin A were not taken at random for the comprehensive investigations here mentioned, it is clear that the recent studies of this kind reveal hitherto unexpected degrees of potentiality in the improvement of already normal health and efficiency through a more scientifically guided daily use of food.

Studies of the human food supplies of the United States, of western and southern Europe, of Russia, of the Orient, and of Puerto Rico, indicate that in all these regions (and doubtless in others as well) the most promising directions for nutritional service to human welfare and progress are those which were found to play the chief parts in the laboratory investigations just outlined.

Very significant too are the results obtained by Orr, Thomson, and Garry (1935) in which, in experiments continued for four generations, half the rats of a colony were fed on a dietary based on what had been found in use in a human population, while the other half received the same diet plus additional milk and green food. This enrichment of the diet resulted in: (1) greater success

in the rearing of young; (2) a markedly decreased death-rate, largely due to lessened incidence of, and better recovery from, an infection to which both halves of the colony were equally exposed; (3) better growth; (4) higher hemoglobin; (5) better appearance as to general condition and sleekness of coat. Orr and his coworkers conclude that more milk and green vegetables in the food supply can very materially improve the physique and positive health of at least a large proportion of the people.

Here and elsewhere there is evidence that, in ways perhaps not yet fully understood, liberal use of protective foods may serve to diminish the incidence, or severity, or duration of other diseases as well as those which are primarily nutritional. Wilder and Boudreau have emphasized the present-day clinical view that the nutritional background and foundation afforded by the habitual use of a dietary which is thoroughly well-balanced, with reference to the newly discovered as well as longer known factors, may greatly advance the probability of a satisfactory outcome of whatever medical measure is to be undertaken. And more recently Sabin and Duffy (1940) report that nutrition is a "factor in the development of constitutional barriers" to certain nervous diseases.

There is also a growing body of scientific evidence that, while physical and mental efficiency do not always go together, yet in the case of a given individual, family, or community, the superior nutritional well-being induced by taking a higher proportion of the needed calories in the form of the protective foods is favorable to the efficiency of the brain as well as of all the other organs. However this may be, the preceding paragraphs of this section afford ample illustrations of the fact that in the well-controlled laboratory-bred colony of experimental animals, used for nutrition investigations extending throughout entire lives and successive generations, in numbers sufficiently large to permit statistical interpretation of findings, we now have an instrument of research such as has not existed before, and which is throwing important light upon previously insoluble problems of the extent to which our life histories are amenable to conscious control and improvement.

### **Deathrates Decreased and Health Enhanced at All Stages of the Life History**

The simple survivorship diagrams shown in Figs. 45 and 46 illustrate both the fact that the same nutritional improvement of

an already adequate diet may decrease deathrates at all ages, and also that some particular enrichment while beneficial throughout life may induce quantitatively greater improvement in a particular segment of the life cycle. Several phases of the experimental evidence have been studied in previous chapters. Now that the

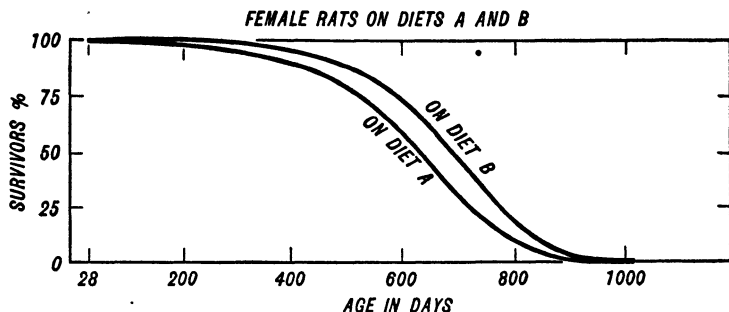


FIG. 45. — Survivorship diagram showing difference in records of parallel groups of rats on Diets A and B respectively.

general principle of the nutritional improvability of the normal has been established with comprehensive convincingness by large numbers of full-life experiments, the way is open for further research planned with reference to the different age periods.

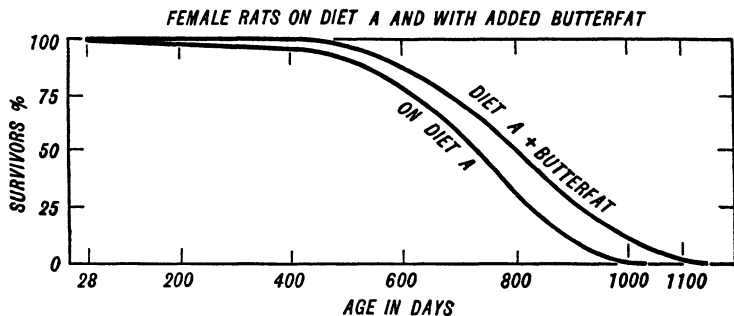


FIG. 46. — Survivorship diagram, comparable with that of Fig. 45, showing records of parallel groups of rats on Diet A and on Diet A plus butterfat (vitamin A) respectively.

In this field of research some of the most fundamental problems are only just now being recognized as amenable to controlled experimentation by the methods of the exact sciences, and the fact that such methods do not at once extend over the whole of this new scientific frontier tends to cause some temporary misappre-

hensions where instead we should be recognizing important opportunities for further research.

Thus current researches upon relations of nutrition to longevity, carried on independently and simultaneously in the laboratory of animal nutrition at Cornell and the laboratory of nutritional chemistry at Columbia, have yielded certain results which at first glance may appear contradictory but which on further examination are readily seen to be consistent. In fact they importantly supplement each other, but do not yet complete each other, for even together they do not yet cover the whole "front" of the new field of exploratory research to which both belong. The finding which at first glance seems confusing is that at Cornell longer life followed retarded growth, while at Columbia increases of growth rate and length of life both resulted from the same improvement of the food supply; but the Cornell and Columbia findings are in harmony when the great difference in the dietary starting-points of the two researches is considered. And the consideration of this difference not only clarifies the interpretation of these particular experiments but also gives a hint of the breadth of significance for human progress, in the understanding and utilization of material resources, which this general field of research appears to offer.

The dietary starting point of the Cornell experiments was a food mixture which had been made extraordinarily rich in proteins and vitamins so that the intakes of these nutrient factors should still be very liberal when the daily allowance of the food mixture was so reduced as to control the rate of growth through the regulation of the food-calorie supply. The extreme richness (with palatability) of this food supply was such as may be approached in occasional cases of the "forcing" of farm animals for maximal gains in body weight; and possibly when an infant is fed without regard to economic considerations and with too great a desire to make a phenomenal growth record. In the nutrition of farm animals the problem of the advisability of "forcing the growth" by *ad libitum* feeding of extremely rich food mixtures may frequently be of economic importance. In human nutrition such problems would arise, if at all, only in the homes of the small minority of families of highest income, or in the minds of pediatricians practicing in correspondingly privileged groups. In contrast, the dietary starting point of the Columbia experiments was representative of the food supplies upon which most people live, with only an economi-

cal proportion of "protective" foods and therefore only about minimal-adequate intakes of several nutritional factors. It was the *Diet A* of the last section of Chapter XXVIII and, as there shown, an increase in its proportion of protective food (or, in chemical terms, simultaneous moderate enrichment in protein, calcium, riboflavin, and vitamin A value) had the effect both of expediting growth and development and of increasing the length of life.

Very significant for human progress is the fact that, as explained in Chapter XXVIII, this capacity to respond was shown even by families that had been obliged to adapt themselves to the minimal-adequate dietary through many generations.

Thus, scientific examination of the above-mentioned Cornell and Columbia findings shows them to be quite consistent when the differences in the plans and starting-points of the respective experiments are considered. If the findings look different at first, it is because they are the results of cultivation of the opposite edges of a very wide field of research. The area which lies between may be quite as fruitful as the areas thus far cultivated. In fact, exploratory work has already shown that different nutritional improvements, of dietaries which are originally suboptimal in several respects, may differ as to the part of the life process upon which they confer their chief benefits. Thus in certain circumstances where increased vitamin A value of the family dietary had no apparent effect upon growth or reproduction it did decrease the adult deathrates and increase the length of life.

Superficial appearances may also be misleading as to the status of evidence regarding the relations of heredity and nutrition to longevity.

Heredity and nutrition both have important influence upon the length of life. The evidence is ample both that heredity, and that nutrition, is a significant factor; but the available research methods are so different for these two factors that the findings do not admit of a scientifically sound answer to any question as to relative importance of heredity and nutrition. The method of research which revealed the influence of heredity upon longevity was not capable of revealing the influence of nutrition; and in the experiments which have demonstrated the influence of nutrition, hereditary factors have been carefully kept uniform. When these facts are understood it is clear that there is here no case of rival hypotheses:

to each method of investigation we are indebted for certain positive findings of fact; and a fact found by one method is not discredited by the limitations of the other. Beside being futile, any attempt to treat the hereditary and nutritional factors in a spirit of rivalry is also ambiguous. *Longevity* literally means simply length of life, but it is also used in the sense of exceptional length of life. Exceptional length of life is doubtless hereditary in some families, as other constitutional tendencies are in other families; and it is also doubtless true that a much greater number of families, while they should not be expected to produce centenarians, do have it in their power to add at least ten per cent to the traditional expectation of seventy years, by making a life-time habit of eating according to the newer knowledge of nutrition.

And the extra years, whatever their number may be, are not to be thought of as added at the end of life, but rather as an extension of the "period of the prime" when both the individual realization of efficiency in accomplishment and the social value of one's services are highest.

The present position of science is that, with full recognition of the important influence of hereditary factors upon the life process, it is now being found that environmental factors are important also. Thus whereas his earlier work had emphasized the hereditary factor, more recently Pearl (1938) has shown clearly that what one takes into the body, even under acceptably normal conditions and habits of living, does very significantly influence the length of human life.

### **Additional Critique of Interpretation**

Both the statistical study of human experience and the use of scientifically chosen species of laboratory animals for controlled experimentation are important in the comprehensive study of the influence of our environments upon our life histories.

In previous chapters something has been said of the detailed studies of nutritional processes in the rat, — notably those of Folin and Morris and of Osborne and Mendel, — which especially encouraged the extensive use of this species as an instrument of research in many (not all) of the problems of human nutrition. In brief the chemistry of the nutrition of the human and rat species is strikingly similar except that the rat is much less dependent than are we upon the vitamin C and nicotinic acid contents of the food.

Inasmuch as our nutritional well-being responds as does that of the rat to the protein, calcium, and riboflavin content, and vitamin A value of the food, and as the human organism is much the more responsive to improvement of the dietary in its vitamin C and nicotinic acid or antipellagric values, it follows that one need not discount the human applicability of the above-described laboratory feeding experiments on the ground of species difference but, on the contrary, that through the chemical studies of the two species it may be seen that the impressions received from experimentation with the rat are *well within* the scientific probabilities of the extent to which the human life process may be improved and length of life extended through the use of the new chemistry of nutrition.

Science had not foreseen the extent to which, as comprehensive and well controlled experiments have now shown, an accepted norm of health can be built to higher levels by nutritional improvement of an already adequate diet. Yet, as briefly indicated in Chapter XXVIII, when these experimental findings of the new chemistry of nutrition are critically examined in the light of physico-chemical principles, they are found to be entirely consistent with the modern teachings and present trend of the exact sciences.

Normality of condition in the body is no longer to be conceived as so rigidly fixed a point, or line, or level as the science of the past two or three generations had supposed; but rather as an area or zone, within which the regulatory processes of the body maintain *relatively* "steady states," *e.g.*, concentration levels and dynamic-equilibrium points within a normal *range*.

From the viewpoint of present-day principles it seems clear enough that within the physiologically normal ranges or zones there must be shifts of concentration levels or dynamic-equilibrium points, or both, when we introduce into our systems different amounts and proportions of such active factors as we know some of the constituents of everyday foods to be. It is a separate research problem to ascertain experimentally, for each nutritional essential, what level of intake has the most favorable effect upon the body's internal environment as judged by observations upon the entire life cycle and successive generations.

Thus chemistry, having investigated the working of the automatic regulatory processes with which we are endowed by our



biological inheritance, is now adding the scientific knowledge which will enable us to make still better use of this bodily endowment. We are finding that certain quantitative levels and relationships in what we take into the body as food, serve to keep the actual conditions of our internal chemistry within the more favorable part of that wider range which the merely automatic working of the regulatory process upon chance intakes permits.

Here the present-day chemistry of nutrition, which is being developed in the light of modern physical and physiological chemistry and by the use (in large numbers and with strictly quantitative laboratory control) of the whole animal organism throughout its whole lifetime as an instrument of research, is now enabling us to add, to the automatic regulation of our biological inheritance, new resources of scientific knowledge and method, for the more precise and permanent attainment and maintenance of such an internal environment as may be found most favorable for the various activities of our lives.

### **Prospective Further Functioning of This New Chemistry of Nutrition**

Within the scientific probabilities which the near future of work in this field seems to offer, there is nothing so dramatic as a mutation, or as some of the exploits of endocrinology; but perhaps there may be something of equally far-reaching significance, and certainly more immediately applicable and controllable by each individual and family who will.

Moreover, as already suggested, it seems probable that the benefits to be induced through following the guidance of the newer chemistry of nutrition in the daily choice and use of food can be added to the benefits realized through the other scientific advances which promise to function for human progress. We do not yet know what the chance variations in human constitution may cover, and so, when we point out the scientific probability that the benefits offered by the newer chemistry of food and nutrition can be added to the benefits which one inherits and to those which one acquires in other ways, this does not necessarily imply that the greatest people who have lived could have been greater still if they had possessed and used our present-day knowledge.

We can, however, quite confidently anticipate that, as it comes

to be generally known and used, the new scientific guidance will bring to a much larger proportion of people such generous measure of buoyant health and capacity for achievement as only the most fortunate few now enjoy.

And the benefit which we may clearly and confidently anticipate is much more than merely biological. It relates not only to health but *through* health to social evolution and to ever higher levels of intellectual and spiritual achievement by the individuals who *will* so to develop their innate capacities.

A satisfactory life requires time.

The shortness of human life, and particularly of that segment of the life cycle in which the fullest functioning is possible, often prevents the individual either from enjoying the realization of as high an aspiration, or from achieving as much in the service of others, as he could with more time. Thus in one of his annual reports, commenting upon the deaths of colleagues, the late President Woodward of the Carnegie Institution of Washington remarked that a third of a professional or scientific man's years have usually passed by the time he has finished his formal schooling and entered his constructive life-work; then probably another third will be spent in proving to himself and to others what he is able to do, before he will be entrusted with his highest responsibilities; and so, only the last third of his years remain in which to render his fullest service to the world.

Recent chartings of the age incidence of major opportunities of presumably representative men in occupations of a scientific or related administrative or educational nature, strikingly confirm Woodward's impression that the most frequent time of attaining one's fullest opportunity is around the age of 50 years. Perhaps equally striking is the wide range of ages at which appreciable numbers of men have actually found (or been admitted to) their major opportunities.

Both these facts emphasize strongly the advantages to the individual and the gains to society which may confidently be anticipated from the earlier attainment and the longer retention of the full adult capacity and efficiency of the people who will have received the benefits of the new knowledge of nutrition.

Such improvements should greatly facilitate, what is now being found so important both in scientific research and in the industrial world, team-work on terms of essential equality between

younger and older people, to bring into the service of a given enterprise the full advantages both of the newer training, and of the more mature experience, as well as of differing but mutually helpful points of view.

Fully to realize the importance of this, it must be constantly kept in mind that when superior nutrition increases the length of life this is only one expression of the fact that the life has been lived on a higher level of health and efficiency throughout. For the individual, it means not more years of old age but more years in the prime of life. For the nation, it means that a larger percentage of the population will be people who are mature and still of full economic, cultural, and social value.

As President Merriam of the Carnegie Institution of Washington pointed out in his essay entitled, "Are the Days of Creation Ended?";\* the direction of human evolution is now largely social, and society is a continuing organism interested in its own future. What promises to affect this future should influence our decisions from day to day and will do so more effectively with the growth of the scientific spirit which expects progress, and works for it; but meanwhile the shortness of individual lives tends to set a limit to the actual use by man of the knowledge which he has accumulated and the institutions which he has built and developed. Hence the longer term of fully efficient years which the newer chemistry of food and nutrition offers may be of exceedingly far-reaching significance to human progress.

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## APPENDIX A

**TABLE 61.—FACTORS FOR CALORIFIC VALUES OF VARIOUS MATERIALS  
WHEN BURNED IN OXY-CALORIMETER**

(Courtesy of Dr. F. G. Benedict and *Industrial and Engineering Chemistry*)

| MATERIAL                                | CALORIES PER LITER OXYGEN |
|---|---------------------------|
| <b>Pure Substances:</b>                 |                           |
| Sucrose . . . . .                       | 5.08                      |
| Lactose . . . . .                       | 5.00                      |
| Benzoic acid <sup>a</sup> . . . . .     | 4.58                      |
| Salicylic acid <sup>a</sup> . . . . .   | 4.65                      |
| Hippuric acid <sup>a</sup> . . . . .    | 4.65                      |
| Uric acid . . . . .                     | 4.55                      |
| <b>Commonly Metabolized Compounds:</b>  |                           |
| Starch . . . . .                        | 5.06                      |
| Dextrose . . . . .                      | 5.01                      |
| Lactic acid . . . . .                   | 4.85                      |
| Animal fat . . . . .                    | 4.72                      |
| Human fat . . . . .                     | 4.79                      |
| Protein . . . . .                       | 4.60                      |
| Acetone . . . . .                       | 4.82                      |
| $\beta$ -Hydroxybutyric acid . . . . .  | 4.85                      |
| Ethyl alcohol . . . . .                 | 4.85                      |
| <b>Foods:</b>                           |                           |
| <b>High carbohydrate materials:</b>     |                           |
| Dried skimmed milk . . . . .            | 4.89                      |
| Oyster crackers . . . . .               | 4.90                      |
| Corn meal . . . . .                     | 4.88                      |
| Nut bread . . . . .                     | 4.88                      |
| Cheese sandwich . . . . .               | 4.95                      |
| Chicken sandwich . . . . .              | 4.95                      |
| Salmon salad sandwich . . . . .         | 4.98                      |
| Club sandwich . . . . .                 | 4.93                      |
| Doughnut . . . . .                      | 4.90                      |
| <b>Highly nitrogenous materials:</b>    |                           |
| "Glidine" (vegetable protein) . . . . . | 4.67                      |
| Ossein . . . . .                        | 4.69                      |
| Collagen . . . . .                      | 4.70                      |
| Plasmon . . . . .                       | 4.65                      |
| <b>Fats:</b>                            |                           |
| Olive oil . . . . .                     | 4.74                      |
| Corn oil . . . . .                      | 4.71                      |
| Cottonseed oil . . . . .                | 4.70                      |
| Cod liver oil . . . . .                 | 4.70                      |

<sup>a</sup> Correction for unburnt carbon necessary.

TABLE 61. — FACTORS FOR CALORIFIC VALUES (*Continued*)

| MATERIAL                   | CALORIES PER LITER OXYGEN |
|----------------------------|---------------------------|
| <i>Foods — Continued</i>   |                           |
| <i>Fats — Continued</i>    |                           |
| Goose fat . . . . .        | 4.75                      |
| Butter . . . . .           | 4.62                      |
| <i>Mixed foods:</i>        |                           |
| Beef stew . . . . .        | 4.84                      |
| Mince pie . . . . .        | 4.97                      |
| <i>Animal foods:</i>       |                           |
| Hay, specimen I . . . . .  | 4.80                      |
| Hay, specimen II . . . . . | 4.86                      |
| Cottonseed meal . . . . .  | 4.66                      |
| Linseed meal . . . . .     | 4.76                      |
| Gluten meal . . . . .      | 4.85                      |
| <i>Excreta:</i>            |                           |
| Human feces . . . . .      | 4.97                      |
| Steer feces . . . . .      | 4.84                      |

## APPENDIX B

### PROXIMATE COMPOSITION AND ENERGY VALUES OF FOODS

Food as purchased may or may not consist entirely of edible material. Meat, for example, may be purchased on the bone; or peas, in the pod. Chatfield and Adams' *Proximate Composition of American Food Materials* and the writer's *Food Products* give data and explanations regarding the kinds and proportions of inedible material in foods as purchased.

In the present book it seems sufficient to give the nutrients in, and the energy or fuel value of, the edible portions of each food listed. Unless otherwise explained, the data contained in Table 62 herewith are based on those of Chatfield and Adams as given in U. S. Dept. Agriculture Circular No. 549, published in June 1940.

From the energy values given in the last two columns of this table, the nutrients shown in this or the subsequent tables, can readily be calculated from the percentage or per 100 gram basis to the basis of the 100-Calorie portion if desired.

TABLE 62. — PROTEIN, FAT, CARBOHYDRATE, AND ENERGY VALUES OF THE  
EDIBLE PORTION OF FOODS

| FOOD               | PROTEIN         | FAT             | CARBO-<br>HYDRATE | FUEL VALUE<br>PER 100 G. | 100-<br>CALORIE<br>PORTION |
|--------------------|-----------------|-----------------|-------------------|--------------------------|----------------------------|
|                    | <i>per cent</i> | <i>per cent</i> | <i>per cent</i>   | <i>Calories</i>          | <i>grams</i>               |
| Almonds            | 18.6            | 54.1            | 19.6              | 640                      | 16                         |
| Apples             | 0.3             | 0.4             | 14.9 <sup>a</sup> | 64                       | 156                        |
| Apricots, fresh    | 1.0             | 0.1             | 12.9 <sup>b</sup> | 56                       | 177                        |
| dried              | 5.2             | 0.4             | 66.9 <sup>c</sup> | 292                      | 34                         |
| Artichokes, French | 2.9             | 0.4             | 11.9              | 63                       | 159                        |
| Asparagus          | 2.2             | 0.2             | 3.9               | 26                       | 381                        |
| Avocado *          | 2.1             | 20.1            | 7.4               | 219                      | 46                         |
| Bacon, fat         | 6.2             | 76.0            | (0.7)             | 712                      | 14                         |
| broiled            | 25.0            | 55.0            | 1.0               | 599                      | 17                         |

\* Average of different varieties as in previous editions of this book.

<sup>a</sup> Includes 0.47% malic acid.

<sup>b</sup> Includes 1.19% malic acid.

<sup>c</sup> Includes 5.0% malic acid.



TABLE 62. — PROXIMATE COMPOSITION OF FOODS (*Continued*)

| FOOD                      | PROTEIN         | FAT             | CARBO-<br>HYDRATE | FUEL VALUE<br>PER 100 G. | 100-<br>CALORIE<br>PORTION |
|---------------------------|-----------------|-----------------|-------------------|--------------------------|----------------------------|
|                           | <i>per cent</i> | <i>per cent</i> | <i>per cent</i>   | <i>Calories</i>          | <i>grams</i>               |
| Bananas                   | 1.2             | 0.2             | 23.0 <sup>d</sup> | 99                       | 101                        |
| Barley, pearled           | 8.2             | 1.0             | 78.8              | 357                      | 28                         |
| whole                     | 12.8            | 2.1             | 72.8              | 361                      | 28                         |
| Beans, baked, with pork   | 5.7             | 2.0             | 19.0              | 117                      | 86                         |
| baked, without pork       | 6.0             | 0.4             | 18.8              | 103                      | 97                         |
| dried, kidney             | 22.0            | 1.5             | 62.1              | 350                      | 29                         |
| Lima, green               | 20.7            | 1.3             | 61.6              | 341                      | 29                         |
| snap or string            | 2.4             | 0.2             | 7.7               | 42                       | 237                        |
| Beef, medium              | 17.5            | 22.0            | — <sup>*</sup>    | 268                      | 37                         |
| loin, medium              | 16.9            | 25.0            | —                 | 293                      | 34                         |
| plate and brisket, medium | 15.8            | 30.0            | —                 | 333                      | 30                         |
| round, lean               | 19.7            | 8.0             | —                 | 151                      | 66                         |
| corned, medium            | 15.8            | 25.0            | —                 | 288                      | 35                         |
| Beets, fresh              | 1.6             | 0.1             | 9.6               | 46                       | 219                        |
| Beet greens               | 2.0             | 0.3             | 5.6               | 33                       | 302                        |
| Blackberries, fresh       | 1.2             | 1.1             | 11.9 <sup>e</sup> | 62                       | 160                        |
| Blueberries, fresh        | 0.6             | 0.6             | 15.1 <sup>f</sup> | 68                       | 147                        |
| Bluefish                  | 20.5            | 4.0             | —                 | 118                      | 85                         |
| Breads:                   |                 |                 |                   |                          |                            |
| Graham, made with milk    | 10.0            | 4.0             | 47.0              | 264                      | 38                         |
| made with some milk       | 9.5             | 3.5             | 48.               | 262                      | 38                         |
| made with water           | 9.0             | 3.0             | 49.               | 259                      | 39                         |
| Vienna bread              | 8.4             | 1.0             | 55.2              | 263                      | 38                         |
| White, milk               | 9.0             | 3.6             | 49.8              | 268                      | 37                         |
| commercial                | 8.5             | 2.0             | 52.3              | 261                      | 38                         |
| Broccoli                  | 3.3             | 0.2             | 5.5               | 37                       | 270                        |
| Brussels sprouts          | 4.4             | 0.5             | 8.9               | 58                       | 173                        |
| Butter                    | 0.6             | 81.0            | 0.4 <sup>e</sup>  | 733                      | 14                         |
| Buttermilk, genuine       | 3.5             | 0.5             | 4.6               | 37                       | 271                        |
| artificially cultured     | 3.5             | 0.2             | 5.0               | 36                       | 279                        |
| Cabbage                   | 1.4             | 0.2             | 5.3               | 29                       | 350                        |
| Cantaloupe                | 0.6             | 0.2             | 5.9               | 28                       | 360                        |
| Carrots                   | 1.2             | 0.3             | 9.3               | 45                       | 224                        |
| Cashew nuts               | 19.6            | 47.2            | 26.4              | 609                      | 16                         |
| Catsup, tomato            | 2.0             | 0.4             | 24.5 <sup>a</sup> | 110                      | 91                         |
| Cauliflower               | 2.4             | 0.2             | 4.9               | 31                       | 323                        |
| Celery                    | 1.3             | 0.2             | 3.7               | 22                       | 459                        |
| Chard (Swiss chard)       | 2.6             | 0.4             | 4.8               | 33                       | 301                        |
| Cheese, Cheddar, American | 23.9            | 32.3            | 1.7               | 393                      | 25                         |
| cottage                   | 19.2            | 0.8             | 4.3               | 101                      | 99                         |
| Parmesan                  | 36.3            | 27.4            | 2.3               | 401                      | 25                         |
| Roquefort                 | 21.7            | 33.2            | 1.4               | 391                      | 25                         |
| Swiss                     | 28.6            | 31.3            | 1.9               | 404                      | 25                         |

<sup>d</sup> Includes 0.39% malic acid.<sup>e</sup> Including 0.91% citric acid.<sup>f</sup> Including 0.67% citric acid.<sup>a</sup> Includes both the lactose and the lactic acid of that part of the buttermilk which the butter retains.<sup>b</sup> Includes 1.5% citric acid.<sup>\*</sup> A dash,—, signifies a negligible value.

TABLE 62. — PROXIMATE COMPOSITION OF FOODS (*Continued*)

| FOOD                                     | PROTEIN         | FAT             | CARBO-<br>HYDRATE | FUEL VALUE<br>PER 100 G. | 100-<br>CALORIE<br>PORTION |
|--|-----------------|-----------------|-------------------|--------------------------|----------------------------|
|  | <i>per cent</i> | <i>per cent</i> | <i>per cent</i>   | <i>Calories</i>          | <i>grams</i>               |
| Cherries, sweet                          | 1.1             | 0.5             | 17.8 <sup>a</sup> | 80                       | 125                        |
| Chestnuts                                | 2.8             | 1.5             | 41.5              | 191                      | 52                         |
| Chicken, total edible                    | 21.6            | 2.7             | —                 | 111                      | 90                         |
| Chives                                   | 3.8             | 0.6             | 7.8               | 52                       | 193                        |
| Chocolate                                | (5.5)           | 52.9            | (18.)             | 570                      | 18                         |
| Clams                                    | 12.8            | 1.4             | 3.4               | 77                       | 129                        |
| Cocoa                                    | (9.)            | 18.8            | (31.)             | 329                      | 30                         |
| Coconut, dried                           | 3.6             | 39.1            | 53.2              | 579                      | 17                         |
| fresh                                    | 3.4             | 34.7            | 14.0              | 382                      | 26                         |
| Cod                                      | 16.5            | 0.4             | —                 | 70                       | 144                        |
| Collards                                 | 3.9             | 0.6             | 7.2               | 50                       | 201                        |
| Corn (maize) entire                      | 10.0            | 4.3             | 73.4              | 372                      | 27                         |
| sweet, fresh                             | 3.7             | 1.2             | 20.5              | 108                      | 93                         |
| canned                                   | 2.5             | 0.9             | 19.6              | 96                       | 104                        |
| Corn flakes                              | 7.9             | 0.7             | 80.3              | 359                      | 28                         |
| Corn meal                                | 9.1             | 3.7             | 73.9              | 365                      | 27                         |
| Crackers, Graham                         | 8.0             | 10.0            | 74.3              | 419                      | 24                         |
| soda, plain                              | 9.6             | 9.6             | 72.7              | 416                      | 24                         |
| Water crackers, unshortened              | 10.7            | 0.3             | 82.1              | 374                      | 27                         |
| Cranberries                              | 0.4             | 0.7             | 11.3 <sup>v</sup> | 53                       | 188                        |
| Cream, light                             | 2.9             | 20.0            | 4.0               | 208                      | 48                         |
| heavy                                    | 2.3             | 35.             | 3.2               | 337                      | 30                         |
| Cress, water                             | 1.7             | 0.3             | 3.3               | 23                       | 441                        |
| Cucumber                                 | 0.7             | 0.1             | 2.7               | 14                       | 690                        |
| "Currants" dried                         | 2.3             | 0.5             | 71.2 <sup>k</sup> | 298                      | 33                         |
| Currants, fresh                          | 1.6             | 0.4             | 12.7 <sup>i</sup> | 61                       | 164                        |
| Currant juice                            | 0.3             | 0.0             | 10.1 <sup>m</sup> | 42                       | 240                        |
| Dandelion greens                         | 2.7             | 0.7             | 8.8               | 52                       | 191                        |
| Dasheens (Taro)                          | 2.9             | 0.2             | 28.9              | 129                      | 77                         |
| Dates                                    | 2.2             | 0.6             | 75.4              | 316                      | 32                         |
| Duck, total edible                       | 16.0            | 28.6            | —                 | 321                      | 31                         |
| Eggplant                                 | 1.1             | 0.2             | 5.5               | 28                       | 355                        |
| Eggs, total edible                       | 12.8            | 11.5            | 0.7               | 158                      | 64                         |
| white only                               | 10.8            | —               | 0.8               | 46                       | 216                        |
| yolk only                                | 16.3            | 31.9            | 0.7               | 355                      | 28                         |
| Endive (escarole)                        | 1.6             | 0.2             | 4.0               | 24                       | 413                        |
| Farina                                   | 11.5            | 1.0             | 76.1              | 359                      | 28                         |
| Figs, dried                              | 4.0             | 1.2             | 68.4 <sup>n</sup> | 300                      | 33                         |
| fresh                                    | 1.4             | 0.4             | 19.6 <sup>o</sup> | 88                       | 114                        |
| Flour (see Wheat)                        |                 |                 |                   |                          |                            |
| Gooseberries                             | 0.8             | 0.4             | 10.1 <sup>p</sup> | 47                       | 212                        |
| Grapefruit                               | 0.5             | 0.2             | 10.1 <sup>q</sup> | 44                       | 226                        |
| Grapefruit juice,<br>canned, unsweetened | 0.4             | 0.1             | 11.1 <sup>r</sup> | 47                       | 213                        |

<sup>a</sup> Includes 0.68% malic acid.<sup>v</sup> Includes 2.36% citric acid.<sup>k</sup> Includes 1.8% malic acid.<sup>i</sup> Includes 2.30% citric acid.<sup>m</sup> Includes 2.00% citric acid.<sup>n</sup> Includes 0.6% malic acid.<sup>r</sup> Includes 1.6% citric acid.<sup>o</sup> Includes 0.17% citric acid.<sup>p</sup> Includes 2.32% citric acid.<sup>q</sup> Includes 1.65% citric acid.

TABLE 62. — PROXIMATE COMPOSITION OF FOODS (*Continued*)

| FOOD                            | PROTEIN         | FAT             | CARBO-<br>HYDRATE | FUEL VALUE<br>PER 100 G. | 100-<br>CALORIE<br>PORTION |
|---------------------------------|-----------------|-----------------|-------------------|--------------------------|----------------------------|
|                                 | <i>per cent</i> | <i>per cent</i> | <i>per cent</i>   | <i>Calories</i>          | <i>grams</i>               |
| Grapefruit juice, sweetened     | 0.4             | 0.1             | 16.1 <sup>a</sup> | 67                       | 149                        |
| Grapes                          | 1.4             | 1.4             | 14.9 <sup>t</sup> | 78                       | 129                        |
| Grape juice                     | 0.4             | —               | 18.5 <sup>w</sup> | 76                       | 132                        |
| Guavas                          | 1.0             | 0.6             | 17.1 <sup>v</sup> | 78                       | 129                        |
| Haddock                         | 17.2            | 0.3             | —                 | 72                       | 140                        |
| Halibut                         | 18.6            | 5.2             | —                 | 121                      | 82                         |
| Hazelnuts                       | 12.7            | 60.9            | 17.7              | 670                      | 15                         |
| Herring, Atlantic               | 19.0            | 6.7             | —                 | 136                      | 73                         |
| Hominy                          | 8.5             | 0.8             | 78.9              | 357                      | 28                         |
| Honey                           | 0.3             | —               | 79.5              | 319                      | 31                         |
| Huckleberries                   | 0.6             | 0.6             | 15.1 <sup>w</sup> | 68                       | 147                        |
| Ice cream, plain                | 3.9             | 13.             | 20.3              | 214                      | 47                         |
| Jams and jellies,<br>commercial | 0.5             | 0.3             | 70.8              | 288                      | 35                         |
| Kale                            | 3.9             | 0.6             | 7.2               | 50                       | 201                        |
| Kohlrabi                        | 2.1             | 0.1             | 6.7               | 36                       | 277                        |
| Lamb, leg, intermediate         | 18.0            | 17.5            | — <sup>*</sup>    | 230                      | 44                         |
| Lemons                          | 0.9             | 0.6             | 8.7 <sup>z</sup>  | 44                       | 228                        |
| Lentils                         | 24.7            | 1.0             | 59.9              | 347                      | 29                         |
| Lettuce                         | 1.2             | 0.2             | 2.9               | 18                       | 549                        |
| Limes                           | 0.8             | 0.1             | 12.3 <sup>y</sup> | 53                       | 188                        |
| Liver (calf)                    | 19.0            | 4.9             | 4.0               | 136                      | 73                         |
| Macaroni                        | 13.0            | 1.4             | 73.9              | 360                      | 28                         |
| Maple sirup (see Sirup)         |                 |                 |                   |                          |                            |
| Milk, fresh whole               | 3.5             | 3.9             | 4.9               | 69                       | 146                        |
| canned, evaporated              | 7.0             | 7.9             | 9.9               | 139                      | 72                         |
| canned, condensed,<br>sweetened | 8.1             | 8.4             | 54.8              | 327                      | 31                         |
| dry, skim                       | 35.6            | 1.0             | 52.0              | 359                      | 28                         |
| dry, whole                      | 25.8            | 26.7            | 38.0              | 496                      | 20                         |
| human                           | 1.4             | 3.7             | 7.2               | 68                       | 148                        |
| Molasses, medium                | —               | —               | (60.)             | 240                      | 417                        |
| Mushrooms                       | (0.)            | 0.3             | (0.)              | — <sup>*</sup>           | — <sup>*</sup>             |
| Muskmelons                      | 0.6             | 0.2             | 5.9               | 28                       | 360                        |
| Oatmeal, dry, uncooked          | 14.2            | 7.4             | 68.2              | 396                      | 25                         |
| Oils, salad                     | —               | 100.            | —                 | 900                      | 11                         |
| Okra                            | 1.8             | 0.2             | 7.4               | 39                       | 256                        |
| Olives, green, pickled          | 1.5             | 13.5            | 4.0               | 144                      | 69                         |
| ripe, pickled                   | 1.6             | 19.0            | 3.0               | 189                      | 53                         |
| Onions                          | 1.4             | 0.2             | 10.3              | 49                       | 206                        |
| Oranges                         | 0.9             | 0.2             | 11.2 <sup>z</sup> | 50                       | 199                        |
| Oysters, fresh, solids          | 9.8             | 2.0             | 5.9               | 81                       | 124                        |
| canned, solids and liquid       | 6.0             | 1.2             | 3.7               | 50                       | 202                        |

<sup>a</sup> Includes 1.6% citric acid.<sup>v</sup> Includes 0.62% citric acid.<sup>y</sup> Includes 5.9% citric acid.<sup>t</sup> Includes 1.21% malic acid.<sup>w</sup> Includes 0.67% citric acid.<sup>z</sup> Includes 0.68% citric acid.<sup>\*</sup> Includes 0.80% malic acid.<sup>\*</sup> Includes 5.07% citric acid.

\* A dash, —, signifies a negligible value.

TABLE 62. — PROXIMATE COMPOSITION OF FOODS (Continued)

| FOOD                      | PROTEIN         | FAT             | CARBO-<br>HYDRATE  | FUEL VALUE<br>PER 100 G. | 100-<br>CALORIE<br>PORTION |
|---------------------------|-----------------|-----------------|--------------------|--------------------------|----------------------------|
|                           | <i>per cent</i> | <i>per cent</i> | <i>per cent</i>    | <i>Calories</i>          | <i>grams</i>               |
| Parsnips                  | 1.5             | 0.5             | 18.2               | 83                       | 121                        |
| Peaches                   | 0.5             | 0.1             | 12.0 <sup>aa</sup> | 51                       | 196                        |
| Peanuts                   | 26.9            | 44.2            | 23.6               | 600                      | 17                         |
| Peanut butter             | 26.1            | 47.8            | 21.0               | 619                      | 16                         |
| Pears                     | 0.7             | 0.4             | 15.8 <sup>bb</sup> | 70                       | 144                        |
| Peas, fresh               | 6.7             | 0.4             | 17.7               | 101                      | 99                         |
| canned                    | 3.3             | 0.2             | 10.1               | 55                       | 181                        |
| dry                       | 23.8            | 1.4             | 60.2               | 349                      | 29                         |
| Pecans                    | 9.4             | 73.0            | 13.0               | 747                      | 13                         |
| Peppers, green            | 1.2             | 0.2             | 5.7                | 29                       | 340                        |
| Persimmons, Japanese      | 0.8             | 0.4             | 20.0 <sup>cc</sup> | 87                       | 115                        |
| native                    | 0.8             | 0.4             | 33.5 <sup>dd</sup> | 141                      | 71                         |
| Pineapple, fresh          | 0.4             | 0.2             | 13.7 <sup>ee</sup> | 58                       | 172                        |
| canned                    | 0.4             | 0.1             | 21.1 <sup>ff</sup> | 87                       | 115                        |
| Pineapple juice, canned   | 0.3             | 0.1             | 13.0 <sup>gg</sup> | 54                       | 185                        |
| Plums                     | 0.7             | 0.2             | 12.9 <sup>hh</sup> | 56                       | 178                        |
| Pork, clear plate, medium | 3.9             | 80.             | —                  | 736                      | 14                         |
| ham, medium               | 15.2            | 31.             | —                  | 340                      | 29                         |
| Potatoes                  | 2.0             | 0.1             | 19.1               | 85                       | 117                        |
| Potato chips              | 6.7             | 37.1            | 49.1               | 557                      | 18                         |
| Prunes, fresh             | 0.9             | 0.2             | 21.8 <sup>ii</sup> | 93                       | 108                        |
| dried                     | 2.3             | 0.6             | 71.0 <sup>jj</sup> | 299                      | 33                         |
| Pumpkin                   | 1.2             | 0.2             | 7.3                | 36                       | 279                        |
| Radishes                  | 1.2             | 0.1             | 4.2                | 22                       | 444                        |
| Raisins                   | 2.3             | 0.5             | 71.2 <sup>kk</sup> | 298                      | 34                         |
| Raspberries               | 1.1             | 0.6             | 14.4 <sup>ll</sup> | 67                       | 148                        |
| Rhubarb                   | 0.5             | 0.1             | 3.8                | 18                       | 552                        |
| Rice, white               | 7.6             | 0.3             | 79.4               | 351                      | 29                         |
| Rye, entire               | 11.2            | 1.7             | 75.2               | 361                      | 28                         |
| Salad dressing—mayonnaise | 1.5             | 78.             | 3.0 <sup>mm</sup>  | 720                      | 14                         |
| Salmon, Pacific, canned   | 20.6            | 9.6             | —                  | 169                      | 59                         |
| Sardines                  | 25.7            | 11.0            | 1.2                | 207                      | 48                         |
| Sausage                   | 11.3            | 41.2            | —                  | 416                      | 240                        |
| Scallops                  | 14.8            | 0.1             | 3.4                | 74                       | 136                        |
| Shad roe                  | 20.9            | 3.8             | (0.)               | 118                      | 85                         |
| Shrimp, canned            | 17.8            | 0.8             | 0.8                | 82                       | 123                        |
| Sirup, cane               | —               | —               | (67.)              | 268                      | 37                         |
| corn, table mixture       | —               | —               | (74.)              | 296                      | 34                         |
| maple                     | —               | —               | (64.)              | 256                      | 39                         |
| Soybeans, fresh           | 12.5            | 6.5             | (6.)               | 132                      | 75                         |
| dry                       | 34.9            | 18.1            | (12.)              | 350                      | 29                         |
| Spinach                   | 2.3             | 0.3             | 3.2                | 25                       | 405                        |
| Squash                    | 1.2             | 0.3             | 7.3                | 37                       | 272                        |

<sup>aa</sup> Includes 0.64% malic acid.<sup>cc</sup> Includes 0.72% citric acid.<sup>ee</sup> Includes 0.98% malic acid.<sup>bb</sup> Includes 0.29% citric acid.<sup>dd</sup> Includes 0.8% citric acid.<sup>ff</sup> Includes 1.7% malic acid.<sup>cc</sup> Includes 0.12% malic acid.<sup>gg</sup> Includes 1.0% citric acid.<sup>hh</sup> Includes 1.8% malic acid.<sup>dd</sup> Includes 0.19% malic acid.<sup>kk</sup> Includes 1.60% malic acid.<sup>ll</sup> Includes 1.34% citric acid.<sup>mm</sup> Includes 0.5% acetic acid.

TABLE 62. — PROXIMATE COMPOSITION OF FOODS (*Continued*)

| FOOD                                | PROTEIN         | FAT             | CARBO-<br>HYDRATE | FUEL VALUE<br>PER 100 G. | 100-<br>CALORIE<br>PORTION |
|-------------------------------------|-----------------|-----------------|-------------------|--------------------------|----------------------------|
|                                     | <i>per cent</i> | <i>per cent</i> | <i>per cent</i>   | <i>Calories</i>          | <i>grams</i>               |
| Strawberries                        | 0.8             | 0.6             | 8.1 <sup>nn</sup> | 41                       | 244                        |
| Sugar, granulated                   | —               | —               | 99.5              | 398                      | 25                         |
| Sweetpotatoes                       | 1.8             | 0.7             | 27.9              | 125                      | 80                         |
| Tapioca                             | 0.6             | 0.2             | 86.4              | 350                      | 29                         |
| Tomatoes                            | 1.0             | 0.3             | 4.0 <sup>oo</sup> | 23                       | 441                        |
| Tomato juice, canned                | 1.0             | 0.2             | 4.3 <sup>pp</sup> | 23                       | 435                        |
| Tongue, medium                      | 16.4            | 15.0            | 0.4               | 202                      | 49                         |
| Tuna, canned                        | 24.2            | 10.8            | —                 | 194                      | 52                         |
| Turkey, medium fat, total<br>edible | 20.1            | 20.2            | —                 | 262                      | 38                         |
| Turnips                             | 1.1             | 0.2             | 7.1               | 35                       | 289                        |
| Turnip tops                         | 2.9             | 0.4             | 5.4               | 37                       | 272                        |
| Veal, medium                        | 19.1            | 12.             | —                 | 184                      | 54                         |
| Walnuts, English                    | 15.0            | 64.4            | 15.6              | 702                      | 14                         |
| Water cress                         | 1.7             | 0.3             | 3.3               | 23                       | 441                        |
| Watermelons                         | 0.5             | 0.2             | 6.9 <sup>aa</sup> | 31                       | 318                        |
| Wheat, entire                       | 11.1            | 1.7             | 75.5              | 362                      | 28                         |
| Wheat flour, Graham                 | 13.             | 2.              | 72.4              | 360                      | 28                         |
| patent                              | 10.8            | 0.9             | 75.9              | 355                      | 28                         |
| Wheat, shredded                     | 10.4            | 1.4             | 78.7              | 369                      | 27                         |

<sup>nn</sup> Includes 1.09% citric acid.<sup>oo</sup> Includes 0.51% citric acid.<sup>pp</sup> Includes 0.4% citric acid.<sup>aa</sup> Includes 0.03% malic acid.

## APPENDIX C

### MINERAL ELEMENTS IN FOODS

#### 1. Calcium, Magnesium, Potassium, Sodium, Phosphorus, Chlorine, Sulfur, and Iron

*The percentage* of each of the eight elements just named is given, for the edible portion of various foods, in Table 63. These figures are the averages of available data, brought as closely and completely as possible up to date (latter part of 1940). Interest in the mineral elements has resulted in the publication of many new data in recent years. The inclusion of these and of new unpublished data from the writer's laboratory, naturally changes many of the averages from those given in previous editions. That the changes (while numerous) are as small as they are is a strong indication that the present data of Table 63 are in most cases nearly true averages. It is, however, to be remembered that foods are subject to *natural variations* in composition, the causes and extents of which are only just beginning to be investigated with adequate thoroughness. Meanwhile there seems to be need to emphasize two facts: (1) that in a balance-experiment the significance of which depends upon precise knowledge of the nutritional intake, the food actually eaten should be analyzed; but (2) that *differences* between the data of such analyses of particular specimens and such averages as those here given are to be taken, assuming accuracy of analytical technique, as *illustrations of natural variability*, and not as necessarily throwing doubt upon the validity of the generally accepted average.

Perhaps it should also be emphasized that *for most dietary and food-supply calculations it is more logical to use averages* of analyses made at different times and on food from different sources (such as those of Table 63) as being more representative of what a given individual or community is apt to get in the long run than the analysis of a specimen or specimens of a single season or source. This is increasingly true as an ever-growing majority of the popu-

lation get more and more of their food through merchandising channels which draw supplies from wide-spread sources.

To indicate the sources of all the data which enter into the averages given in Table 63 would require a prohibitive amount of space.

TABLE 63. — PERCENTAGES OF CERTAIN OF THE MINERAL ELEMENTS IN THE EDIBLE PORTIONS OF FOODS

|                                 | CAL-<br>CIUM       | MAG-<br>NE-<br>SIUM | PO-<br>TAS-<br>SIUM | SO-<br>DIUM        | PHOS-<br>PHO-<br>RUS | CHLO-<br>RINE      | SUL-<br>FUR | IRON    |
|---------------------------------|--------------------|---------------------|---------------------|--------------------|----------------------|--------------------|-------------|---------|
| Almonds                         | .254               | .252                | .759                | .026               | .475                 | .020               | .150        | .0044   |
| Apples                          | .007               | .006                | .116                | .010               | .011                 | .004               | .005        | .0003   |
| Apricots, dried                 | (.071)             | *                   | *                   | *                  | (.113)               | *                  | *           | .0076   |
| Apricots, fresh                 | .015               | .009                | .279                | .030               | .024                 | .002               | .006        | .0005   |
| Artichokes, French              | .039               | .027                | ?                   | .025               | .087                 | .057               | .020        | .0010   |
| Asparagus                       | .021               | .012                | .187                | .016               | .052                 | .036               | .046        | .0012   |
| Avocado                         | .019               | .041                | .653                | .067               | .046                 | .016               | .037        | .0014   |
| Bacon, 10-15%<br>protein        | .012               | .013                | .239                | .820               | .109                 | 1.251              | .152        | .0015   |
| Banana                          | .008               | .031                | .373                | .042               | .028                 | .125               | .012        | .0006   |
| Barley, entire                  | .075               | .171                | .485                | .077               | .373                 | ?                  | .143        | .0051   |
| pearled                         | .016               | .037                | .110                | .056               | .189                 | ?                  | .116        | (.002)  |
| Beans, dried                    | .148               | .159                | 1.201               | .103               | .463                 | .035               | .237        | .0103   |
| Lima, dried                     | .072               | .181                | 1.727               | .167               | .380                 | .031               | .178        | .0090   |
| Lima, fresh                     | .031               | *                   | *                   | *                  | .112                 | *                  | *           | .0023   |
| snap or string                  | .065               | .026                | .251                | .023               | .044                 | .033               | .030        | .0011   |
| Beef, lean                      | .013               | .024                | .338                | .084               | .204                 | .076               | .230        | .0030   |
| Beet                            | .026               | .023                | .336                | .079               | .039                 | .061               | .017        | .0009   |
| Beet greens                     | .134               | .113                | *                   | *                  | .039                 | *                  | *           | .0032   |
| Blackberries, seeds<br>included | .032               | .024                | .181                | .004               | .032                 | .015               | .017        | .0009   |
| seeds removed                   | .017               | *                   | *                   | *                  | .019                 | .007               | .008        | (.0009) |
| Blueberries                     | .026               | .010                | .065                | .016               | .020                 | .008               | .011        | .0009   |
| Bluefish                        | .023               | .031                | .315                | .068               | .235                 | .076               | .241        | .0010   |
| Brazil nuts                     | .124               | .225                | .601                | .026               | .602                 | .081               | .198        | .0028   |
| Bread, white                    | (.05) <sup>a</sup> | .030                | .109                | .446               | (.10) <sup>b</sup>   | .621               | .054        | .0009   |
| whole wheat                     | (.06) <sup>a</sup> | (.15)               | (.45)               | <sup>a</sup>       | (.37)                | <sup>a</sup>       | (.15)       | .0030   |
| Broccoli, E. P.                 | .146               | .029                | .395                | .052               | .072                 | .097               | .145        | .0014   |
| flowerbuds                      | .101               | .031                | .408                | .024               | .107                 | *                  | *           | *       |
| leaves                          | .314               | .041                | .374                | .064               | .066                 | *                  | *           | .0024   |
| twigs                           | .073               | .021                | .361                | .031               | .038                 | *                  | *           | *       |
| Brussels sprouts                | .025               | *                   | *                   | *                  | .105                 | *                  | .184        | .0011   |
| Butter                          | .016               | .001                | .014                | (.22) <sup>c</sup> | .016                 | (.33) <sup>c</sup> | .009        | .0002   |
| Cabbage, headed                 | .045               | .012                | .294                | .032               | .028                 | .039               | .067        | .0004   |
| loose leaf, outer               |                    |                     |                     |                    |                      |                    |             |         |
| leaves, or greens               | .429               | .034                | .402                | .065               | .072                 | .108               | (.07)       | .0018   |
| general average                 | *                  | *                   | *                   | *                  | *                    | *                  | *           | .0007   |

\* Doubtless present but quantitative data have not been found

? Reports too discordant to average.

N. B. Data enclosed in parentheses are based on evidence either less consistent or less direct than in the majority of cases.

<sup>a</sup> Uncertain because of varying methods of breadmaking.

<sup>b</sup> Varies for the same reason, but in lesser degree.

<sup>c</sup> Varies with the amount of added salt.

TABLE 63. — PERCENTAGES OF MINERAL ELEMENTS IN FOOD (*Continued*)

|                   | CAL-<br>CIUM      | MAG-<br>NE-<br>SIUM | PO-<br>TAS-<br>SIUM | SO-<br>DIUM       | PHOS-<br>PHO-<br>RUS | CHLO-<br>RINE     | SUL-<br>FUR | IRON   |
|-------------------|-------------------|---------------------|---------------------|-------------------|----------------------|-------------------|-------------|--------|
| Cantaloupe        | .017              | .017                | .249                | .043              | .016                 | .040              | .015        | .0004  |
| Carrots           | .042              | .017                | .311                | .076              | .040                 | .042              | .021        | .0007  |
| Cashew nuts       | .048              | .267                | *                   | *                 | .480                 | *                 | *           | *      |
| Cauliflower       | .025              | .020                | .313                | .041              | .065                 | .031              | .085        | .0009  |
| Celery            | .072              | .027                | .291                | .130              | .046                 | .137              | .022        | .0007  |
| Chard             | .104 <sup>d</sup> | .053                | .318                | .086              | .050                 | .039              | .124        | .0031  |
| Cheese, hard      | .873              | .042                | .131                | .88 <sup>e</sup>  | .610                 | 1.35 <sup>e</sup> | .218        | (.001) |
| cottage           | .082              | *                   | *                   | *                 | .263                 | *                 | *           | (.001) |
| Cherries          | .017              | .014                | .246                | .003              | .022                 | .003              | .008        | .0005  |
| Chestnuts         | .034              | .042                | .529                | .038              | .090                 | .011              | .048        | .0008  |
| Chicken (fowl)    | .016              | .027                | .372                | .091              | .218                 | .079              | .252        | .0019  |
| Chocolate         | .095              | ?                   | .442                | .056              | .343                 | .071              | .095        | .0025  |
| Clams             | .102              | .089                | .172                | .603              | .105                 | 1.065             | .219        | *      |
| Cocoa             | .112              | .420                | .900                | .059              | .709                 | .051              | .203        | .0027  |
| Coconut, dried    | .043              | .077                | .693                | .053              | .191                 | .225              | .076        | .0036  |
| fresh             | .021              | .039                | .363                | .039              | .098                 | .122              | .032        | .0020  |
| milk              | .024              | ?                   | ?                   | .058              | .029                 | .190              | .032        | .0001  |
| Codfish           | .014              | .022                | .339                | .096              | .188                 | .150              | .203        | .0015  |
| Collards          | .202              | *                   | *                   | *                 | .074                 | *                 | *           | .0016  |
| Conch             | .089              | .246                | *                   | *                 | .112                 | *                 | .315        | .0012  |
| Corn (maize)      | .029              | .121                | .339                | .036              | .281                 | .045              | .151        | .0036  |
| meal              | .016              | .084                | .213                | .039              | .152                 | .146              | .111        | .0009  |
| sweet             | .009              | .038                | .113                | .040              | .120                 | .014              | .046        | .0005  |
| Cranberries       | .014              | .007                | .080                | .006              | .011                 | .005              | .007        | .0006  |
| Cream             | (.09)             | (.01)               | (.13)               | (.03)             | (.07)                | (.08)             | (.03)       | .0002  |
| Cucumbers, seeds  |                   |                     |                     |                   |                      |                   |             |        |
| included          | .010              | .009                | .140                | .010              | .021                 | .030              | .012        | .0003  |
| seeds removed     | .006              | *                   | *                   | *                 | .018                 | *                 | *           | .0003  |
| Currants, dried   | .075              | .030                | .458                | .018              | .138                 | .029              | ?           | .0027  |
| fresh             | .035              | .015                | .261                | .007              | .036                 | .013              | .029        | .0009  |
| Currant juice     | .016              | .010                | .185                | (.006)            | .013                 | .004              | .005        | *      |
| Dandelion         | .113              | .036                | .461                | .168              | .041                 | .099              | .17         | .0030  |
| Dates             | .072              | .065                | .675                | .097              | .060                 | .283              | .065        | .0021  |
| Eggplant          | .009              | .015                | .229                | .015              | .020                 | .047              | .021        | .0005  |
| Eggs              | .058              | .013                | .138                | .140              | .224                 | .120              | .197        | .0031  |
| Egg white         | .011              | .011                | .154                | .170              | .015                 | .161              | .208        | .0001  |
| Egg yolk          | .157              | .016                | .118                | .056              | .538                 | .124              | .194        | .0087  |
| Endive and        |                   |                     |                     |                   |                      |                   |             |        |
| Escarole          | .074              | .013                | .381                | .060              | .038                 | .071              | .032        | .0017  |
| Farina            | .021              | .025                | .120                | .065              | .125                 | .076              | .155        | .0008  |
| Figs, dried       | .223              | .082                | .990                | .066              | .104                 | .105              | .069        | .0031  |
| fresh             | .050              | .021                | .297                | .007              | .035                 | .016              | .012        | .0007  |
| Fish <sup>e</sup> |                   |                     |                     |                   |                      |                   |             |        |
| Flounder          | .031              | .025                | .311                | .107              | .197                 | .151              | .217        | .0010  |
| Flour, buckwheat  | .010              | .048                | .130                | .027              | .176                 | .012              | .071        | .0012  |
| Graham or entire  |                   |                     |                     |                   |                      |                   |             |        |
| wheat             | .035              | .122                | .324                | .160 <sup>f</sup> | .300                 | .177 <sup>f</sup> | .124        | .0040  |
| white             | .015              | .021                | .130                | .045              | .101                 | .071              | .109        | .0013  |
| Gooseberries      | .022              | .009                | .149                | .010              | .028                 | .009              | .015        | .0005  |

<sup>d</sup> Calcium of chard is of very doubtful availability.<sup>e</sup> Average fish is estimated to contain per 100 grams of protein as follows: 0.109 gram Ca; 0.133 gram Mg; 1.671 grams K; 0.373 gram Na; 1.148 grams P; 0.528 gram Cl; 1.119 grams S; 0.0055 gram Fe.<sup>f</sup> Probably contained some added salt.



TABLE 63. — PERCENTAGES OF MINERAL ELEMENTS IN FOOD (*Continued*)

|                      | CAL-<br>CIUM      | MAG-<br>NE-<br>SIUM | PO-<br>TAS-<br>SIUM | SO-<br>DIUM        | PHOS-<br>PHO-<br>RUS | CHLO-<br>RINE       | SUL-<br>FUR | IRON               |
|----------------------|-------------------|---------------------|---------------------|--------------------|----------------------|---------------------|-------------|--------------------|
| Grapefruit           | .017              | .010                | .198                | .004               | .018                 | .003                | .008        | .0003              |
| juice                | .010              | .008                | .139                | .005               | .017                 | .002                | .005        | .0002              |
| Grapes               | .017              | .007                | .254                | .011               | .021                 | .002                | .009        | .0006              |
| Haddock              | .040              | .026                | .314                | (.66) <sup>c</sup> | .200                 | (1.07) <sup>c</sup> | .238        | .0007              |
| Halibut              | .011              | .024                | .340                | .111               | .209                 | .088                | .212        | .0007              |
| Ham, med.-lean       | .022              | .020                | .383                | c                  | .151                 | c                   | .225        | .0022              |
| Hazelnuts            | .287              | .140                | .618                | .019               | .354                 | .067                | .198        | .0041              |
| Heart                | .010              | .035                | .370                | .153               | .236                 | .125                | .296        | .0062              |
| Hominy               | .011              | .058                | .174                | ?                  | .070                 | .046                | ?           | (.001)             |
| Honey                | .005              | .006                | ?                   | .005               | .016                 | .019                | .005        | .0009              |
| Huckleberries        | .026              | .010                | .065                | .016               | .020                 | .008                | .011        | .0009              |
| Kale                 | .181              | .037                | .387                | .052               | .067                 | .122                | .115        | .0025              |
| Kidney               | .016              | .021                | .238                | .230               | .287                 | .246                | .190        | .0065              |
| Kohlrabi             | .078              | .037                | .371                | .050               | .057                 | .053                | .050        | .0007              |
| Lamb (Mutton)        | .015              | .024                | .301                | .084               | .208                 | .085                | .211        | .0030              |
| Lemon (or juice)     | .021              | .009                | .148                | .013               | .012                 | .004                | .008        | .0003              |
| Lentils, dry         | .098              | .086                | .835                | .057               | .368                 | .060                | .277        | .0083              |
| Lettuce <sup>d</sup> | .054 <sup>b</sup> | .011                | .311                | .030               | .031                 | .073                | .018        | .0011 <sup>b</sup> |
| Liver                | .008              | .022                | .298                | .087               | .373                 | .101                | .251        | .0121              |
| Loganberries, fresh  |                   |                     |                     |                    |                      |                     |             |                    |
| and canned           | .027              | .018                | .177                | .002               | .024                 | .011                | .011        | .0021              |
| Macaroni             | .021              | .034                | .174                | .018               | .147                 | .052                | .146        | .0013              |
| Mackerel             | .015              | .033                | .418                | .153               | .261                 | .152                | .197        | .0011              |
| Maple sirup          | .163              | .019                | .242                | .011               | .014                 | .028                | .004        | *                  |
| Meat <sup>e</sup>    |                   |                     |                     |                    |                      |                     |             |                    |
| Milk, cow's          | .118              | .012                | .143                | .051               | .093                 | .106                | .034        | .0002              |
| Molasses**           | .246              | .081                | 1.238               | .043               | .034                 | .501                | .050        | .0093              |
| Mushrooms            | .014              | .016                | .384                | .027               | .098                 | .021                | .051        | .0007              |
| Muskmelon            | .017              | .017                | .249                | .043               | .016                 | .040                | .015        | .0004              |
| Mutton               | .015              | .024                | .301                | .084               | .208                 | .085                | .211        | .0030              |
| Oatmeal (Oats)       | .081              | .145                | .431                | .071               | .365                 | .049                | .199        | .0052              |
| Okra, seeds included | .072              | .038                | *                   | *                  | .062                 | *                   | .014        | .0007              |
| seeds removed        | .075              | .043                | *                   | *                  | .053                 | *                   | *           | .0007              |
| Olives <sup>f</sup>  | .101              | .012                | .809                | 1.189 <sup>c</sup> | .015                 | 1.877 <sup>c</sup>  | .032        | .0020              |
| Onions               | .032              | .015                | .183                | .015               | .044                 | .024                | .068        | .0005              |
| Orange (or juice)    | .025              | .010                | .181                | .010               | .019                 | .004                | .008        | .0003              |
| Oysters              | .068              | .039                | .204                | .471               | .172                 | .628                | .180        | .0071              |
| Parsley              | .193              | *                   | *                   | *                  | .084                 | *                   | *           | .0043              |
| Parsnips             | .057              | .029                | .417                | .008               | .080                 | .035                | .026        | .0007              |
| Peaches              | .009              | .011                | .256                | .015               | .018                 | .005                | .007        | .0003              |
| Peanuts              | .066              | .167                | .614                | .039               | .392                 | .041                | .226        | .0019              |
| Pears                | .013              | .009                | .129                | .008               | .016                 | .004                | .007        | .0003              |
| Peas, dry            | .073              | .140                | .979                | .089               | .397                 | .044                | .196        | .0060              |
| fresh                | .022              | .027                | .284                | .019               | .122                 | .033                | .056        | .0019              |

<sup>a</sup> Though several investigators have published at least partial analyses, the evidence available at time of writing does not show how far the varieties of lettuce differ in composition.

<sup>b</sup> Higher in loose-leaf than in headed lettuce.

<sup>c</sup> Average meat is estimated to contain per 100 grams protein as follows: 0.058 gram Ca; 0.118 gram Mg; 1.694 grams K; 0.421 gram Na; 1.078 grams P; 0.378 gram Cl; 1.146 grams S; 0.0150 gram Fe.

<sup>d</sup> Pickled in brine.

<sup>e</sup> The figures here given for molasses, based on findings reported by Sheets and Pearson (Mississippi Agr. Expt. Sta., Tech. Bull. No. 22, 1936) are probably applicable only to the extreme type of "genuine old-fashioned molasses of the deep South." The composition of what is usually called molasses, throughout the United States generally, is probably more nearly approximated by the data for Sirups as listed farther on in this table.

TABLE 63. — PERCENTAGES OF MINERAL ELEMENTS IN FOOD (*Continued*)

|                     | CAL-<br>CIUM      | MAG-<br>NE-<br>SIUM | PO-<br>TAS-<br>SIUM | SO-<br>DIUM | PHOS-<br>PHO-<br>RUS | CHLO-<br>RINE | SUL-<br>FUR | IRON    |
|---------------------|-------------------|---------------------|---------------------|-------------|----------------------|---------------|-------------|---------|
| Pecans              | .089              | .152                | *                   | *           | .335                 | .050          | .113        | .0026   |
| Pepper, green       | .011              | .012                | .186                | *           | .025                 | .019          | .019        | .0004   |
| Persimmons          | .022              | .009                | .292                | .011        | .021                 | .002          | .005        | .0003   |
| Pineapple           | .016              | .011                | .214                | .014        | .011                 | .046          | .007        | .0003   |
| Plums               | .017              | .011                | .232                | .004        | .020                 | .002          | .005        | .0005   |
| Pork, med.-lean     | .010              | .024                | .304                | .069        | .215                 | .069          | .206        | .0022   |
| Pork (10% protein)  | .006              | .012                | .169                | .042        | .108                 | .038          | .115        | .0015   |
| Potatoes            | .013              | .027                | .496                | .024        | .053                 | .035          | .029        | .0011   |
| Prunes, dry         | .062              | .040                | .848                | .078        | .093                 | .009          | .028        | .0035   |
| Pumpkins            | .021              | .012                | .457                | .054        | .044                 | .049          | .013        | .0008   |
| Radishes            | .037              | .015                | .229                | .064        | .031                 | .037          | .031        | .0010   |
| Raisins             | .055              | .035                | .708                | .087        | .110                 | .045          | .042        | .0030   |
| Raspberries         |                   |                     |                     |             |                      |               |             |         |
| seeds included      | .040              | .023                | .190                | .003        | .037                 | .022          | .018        | .0009   |
| seeds removed       | .024              | .020                | (.14)               | (.04)       | .027                 | *             | (.01)       | (.0009) |
| Raspberry juice     | .024              | .016                | .134                | .005        | .012                 | *             | .009        | (.0008) |
| Rhubarb             | .051              | .016                | .358                | .017        | .025                 | .053          | .008        | .0005   |
| Rice, entire        | .068              | .119                | .342                | .078        | .336                 | .023          | ?           | ?       |
| white               | .009              | .028                | .079                | .028        | .092                 | .006          | ?           | .0007   |
| Rye, entire         | .061              | .155                | (.45)               | .061        | .369                 | (.04)         | .146        | .0048   |
| flour               | .018              | .081                | (.45)               | .019        | .278                 | (.04)         | .134        | .0013   |
| Salmon              | *                 | .029                | .316                | ?           | .289                 | ?             | .226        | .0009   |
| Shrimps             | .075              | .074                | .404                | c           | .210                 | c             | ?           | .0020   |
| Sirups <sup>1</sup> | (.04)             | (.01)               | (.24)               | ?           | (.014)               | .042          | ?           | (.004)  |
| Soybean flour       | .216              | .223                | ?                   | ?           | .583                 | .024          | (.3)        | (.0027) |
| Spinach             | .083 <sup>m</sup> | .055                | .489                | .084        | .048                 | .065          | .027        | .0034   |
| Squash, summer      |                   |                     |                     |             |                      |               |             |         |
| seeds removed       | .015              | .008                | .150                | .002        | .015                 | *             | *           | .0004   |
| winter, seeds       |                   |                     |                     |             |                      |               |             |         |
| removed             | .019              | .011                | .320                | .004        | .028                 | *             | *           | .0006   |
| Strawberries        | .022              | .012                | .145                | .007        | .022                 | .011          | .012        | .0009   |
| Sweetpotato         | .033              | .024                | .373                | .027        | .052                 | .085          | .026        | .0008   |
| Tapioca             | .012              | .002                | .020                | .004        | .012                 | .016          | .004        | (.001)  |
| Tomatoes            |                   |                     |                     |             |                      |               |             |         |
| seeds included      | .011              | .012                | .268                | (.02)       | .027                 | (.04)         | .014        | (.0006) |
| seeds removed       | .006              | .010                | .229                | (.02)       | .020                 | (.04)         | .008        | .0006   |
| Tomato juice        | .007              | .010                | .310                | .015        | .015                 | .055          | .005        | (.0004) |
| Turkey              | .023              | .028                | .367                | .130        | .320                 | .123          | .234        | .0038   |
| Turnip(s)           | .051              | .016                | .327                | .066        | .032                 | .052          | .058        | .0005   |
| Turnip tops         | .254              | .019                | .307                | .045        | .058                 | .092          | .054        | .0035   |
| Veal, med.-lean     | .012              | .023                | .359                | .089        | .221                 | .077          | .203        | .0024   |
| Vinegar             | .016              | .011                | .150                | .020        | .015                 | .047          | .018        | .0004   |
| Walnuts             | .083              | .134                | .525                | .023        | .380                 | .036          | .146        | .0021   |
| Watercress          | .168              | .028                | .301                | .080        | .041                 | .109          | .147        | .0026   |
| Watermelon          | .007              | .010                | .121                | .020        | .012                 | .008          | .009        | .0002   |
| Wheat, entire       | .057              | .165                | .465                | .060        | .374                 | .049          | .171        | .0057   |
| Wine (average)      | .009              | .007                | .104                | .008        | .015                 | .002          | .015        | (.0003) |

\* The calcium content of the edible flesh of fresh salmon, carefully freed from bone, averages about 0.015 per cent. Canned salmon, however, ordinarily includes the bone, and according to the U. S. Department of Commerce, this should be reckoned as consumable and nutritionally available. With bone thus included, the calcium content of salmon averages about 0.194 per cent.

<sup>1</sup> Data here given are averaged from analyses of sirups of several types commonly sold for use as table sirups and in cooking. Such sirups are often called molasses. The differences in mineral composition, both between the different kinds of sirups and between sirup and molasses, are relatively large.

<sup>m</sup> Not nutritionally available.

## 2. Copper and Manganese

*Parts per million* of copper and manganese in the edible portions of typical foods are tabulated, as averages of the data obtained from indicated sources, in Table 64.

In general, the averages in this table do not rest on such numerous data as those in Table 63, and in most cases come from fewer sources. Hence for each average given there are indicated the number of cases and the names of the investigators who reported the data which have been used in obtaining the average.

TABLE 64. — PARTS PER MILLION OF COPPER AND MANGANESE IN THE EDIBLE PORTION OF FOODS

| Food                         | COPPER       |         |                   | MANGANESE    |         |                           |
|------------------------------|--------------|---------|-------------------|--------------|---------|---------------------------|
|                              | No. of Cases | Average | Reference Numbers | No. of Cases | Average | Reference Numbers         |
| Alfalfa, dry                 | 4            | 10.68   | 1                 | 7            | 54.14   | 2                         |
| Apples                       | 26           | .71     | 3,7,8,9,10        | 13           | .84     | 5,6                       |
| Asparagus                    | 5            | 1.41    | 3,4,13,14,15      | 2            | 1.9     | 6,13                      |
| Avocado                      | 17           | 6.88    | 9,16,17           | 47           | 2.91    | 16,17                     |
| Bananas                      | 4            | 2.00    | 3,7,9,18          | 2            | 6.42    | 6,19                      |
| Barley, entire               | 14           | 7.53    | 1,7,20,21,22      | 10           | 16.84   | 2,6,19,23,24, 25          |
| Beans, dry                   | 21           | 9.57    | 1,3,7,9,26,27     | 12           | 15.03   | 6,19,26                   |
| Beans, Lima, dry             | 4            | 9.15    | 3,9,26,27         | 2            | 16.55   | 6,26                      |
| Beans, snap or string        | 7            | 1.26    | 4,13,26,28,29     | 26           | 3.26    | 12,13,26,28, 29,30        |
| Beets                        | 6            | 1.87    | 3,4,7,13,28       | 6            | 5.77    | 6,13,19,28,29             |
| Bread, white                 | 2            | 2.05    | 3,4               | 1            | 3.1     | 12                        |
| Cabbage                      | 9            | .99     | 3,7,9,13,28, 32   | 33           | 1.14    | 6,13,19,28,29, 30, 31, 32 |
| Cantaloupe                   | 3            | .57     | 3,4               | 1            | .42     | 12                        |
| Carrots                      | 7            | 1.11    | 1,3,9,13,28       | 6            | 2.47    | 6,13,19,28,29             |
| Corn (maize)                 | 6            | 4.49    | 1,7,20,27         | 10           | 6.83    | 2,6,19,20,33              |
| Eggs                         | 6            | 2.53    | 3,4,34            | 3            | .33     | 12,19                     |
| Flour, white                 | 3            | 1.47    | 3,4,7             | 3            | 7.13    | 12,25                     |
| Flour, whole wheat or Graham | 2            | 4.35    | 3,4               | 1            | 42.8    | 12                        |
| Grapes                       | 4            | .98     | 3,7,9             | 2            | .83     | 6                         |
| Haddock                      | 19           | 2.16    | 3,34,35,36        | 1            | .15     | 35                        |
| Halibut                      | 3            | 1.60    | 3,4,35            | 1            | .10     | 35                        |
| Honey                        | 5            | .75     | 3,4,38            | 4            | 2.09    | 37,38                     |
| Kale                         | 5            | 3.28    | 39                | 6            | 5.87    | 33,39                     |
| Lettuce, headed              | 9            | .69     | 1,3,9,13,14       | 8            | 7.77    | 6,12,13,19,39, 40         |
| Lettuce, loose leaf          | 9            | 1.45    | 3,28,40           | 9            | 12.40   | 12,28,40                  |

TABLE 64. — PARTS PER MILLION OF COPPER AND MANGANESE  
IN FOODS (*Continued*)

| Food               | COPPER       |         |                             | MANGANESE    |         |                       |
|--------------------|--------------|---------|-----------------------------|--------------|---------|-----------------------|
|                    | No. of Cases | Average | Reference Numbers           | No. of Cases | Average | Reference Numbers     |
| Liver'             | 26           | 34.51   | 4,25,41,42                  | 5            | 3.41    | 19,25                 |
| Mackerel           | 3            | 2.30    | 3,35,36                     | 2            | .20     | 12,35                 |
| Milk               | 87           | .35     | 3,41,43,44,45,<br>46,47,48  | 5            | .06     | 12,19,25,49           |
| Oats (oatmeal)     | 14           | 7.38    | 1,3,4,7,21                  | 9            | 49.45   | 2,6,12,19,24,<br>25   |
| Onions             | 6            | 1.30    | 3,4,9,13,28                 | 7            | 3.63    | 6,13,19,28,29         |
| Orange (or juice)  | 7            | .76     | 3,9,51                      | 1            | .25     | 50                    |
| Oysters            | 54           | 36.23   | 3,36,52,53,54               | 49           | 2.95    | 53,54                 |
| Pears              | 25           | 1.34    | 3,9,10,55                   | 1            | .64     | 6                     |
| Peas (dried)       | 4            | 8.02    | 3,9,27                      | 2            | 19.90   | 12,25                 |
| Peppers (green)    | 3            | 1.07    | 3,28                        | 3            | 1.26    | 6,12,28               |
| Plums              | 6            | .80     | 3,9,56                      | 3            | .96     | 12,56                 |
| Potatoes           | 51           | 1.64    | 3,9,13,28,57                | 50           | 1.73    | 6,13,19,28,29,<br>58  |
| Prunes (dried)     | 17           | 2.91    | 3,9,59                      | 16           | 4.36    | 6,59                  |
| Rice (white)       | 3            | 1.83    | 3,4,26                      | 9            | 10.14   | 2,12,19,23,<br>25,26  |
| Rye (entire grain) | 4            | 6.56    | 1,7,22,26                   | 7            | 30.67   | 2,6,24,26,33          |
| Spinach            | 14           | 1.97    | 3,4,13,14,32,<br>39         | 12           | 8.28    | 6,13,19,29,<br>32,39  |
| Strawberries       | 2            | .75     | 3,9                         | 29           | 2.19    | 6,60                  |
| Sweetpotatoes      | 4            | 1.84    | 3,4,13,28                   | 7            | 4.07    | 6,13,19,28            |
| Tomatoes           | 49           | .97     | 1,3,9,13,14,<br>28,61,62,63 | 17           | 1.89    | 12,13,25,28,<br>30,63 |
| Turnips            | 8            | .85     | 3,4,13,28                   | 6            | .83     | 12,13,19,28,<br>29    |
| Wheat              | 50           | 7.87    | 1,7,21,22,26,<br>64         | 22           | 45.91   | 2,6,19,24,26,<br>33   |

1. Elvehjem and Hart 2. Skinner and Peterson. 3. Lindow, Elvehjem, and Peterson. 4. McCance and Widdowson. 5. Hopkins and Gourley. 6. Lindow and Peterson. 7. Guerithault. 8. Ausbacher, Remington, and Culp. 9. McCance, Widdowson and Shackleton. 10. Jones and Hatch. 11. Colby and Dyer. 12. Peterson and Skinner. 13. Remington and Shiver. 14. Levine, Culp, and Anderson. 15. Flyler and Manchesian. 16. Weatherby and Haas. 17. Haas. 18. Eddy. 19. Richards. 20. McHargue and Roy. 21. Greaves and Anderson. 22. Kogan and Nasyrova. 23. Newcomb and Sankaran. 24. Headden. 25. McHargue. 26. McHargue and Roy. 27. Satterfield and Jones. 28. Coleman and Ruprecht. 29. Munger and Peterson. 30. Peterson and Lindow. 31. Ripperton and Russell. 32. Culp and Copenhagen. 33. Davidson and Capen. 34. Crooks and Ritchie. 35. Parks and Rose. 36. Nilson and Coulson. 37. Elser. 38. Schuette and Remy. 39. Davidson and LeClerc. 40. Wendt and Wilkinson. 41. Grendel. 42. Powick and Hoagland. 43. Boober. 44. Supplee and Bellis. 45. Gamble, Ellis, and Besley. 46. Tompsett. 47. Conn, Johnson, Trebler, and Karpenko. 48. Bing, Saurwein, and Myers. 49. Büttner and Miermeister. 50. Hodges and Peterson. 51. Rabinowitsch. 52. Whipple. 53. Levine, Remington, and Culp. 54. Coulson, Levine, and Remington. 55. Bullis. 56. Andersen. 57. Peterson and Schwogler. 58. Peterson and Hof. 59. Saywell, Dietz, and Robertson. 60. Knight and Wallace. 61. McLean. 62. Englis. 63. Lewis and Marmoy. 64. Webster and Jansma.

## APPENDIX D

### VITAMIN VALUES OF FOODS

Quantitative measurement of vitamin values of foods is a field of work of only recent development and only still more recently have the pure substances been available to permit of the use of present-day methods of determination and expression. There are also differences of opinion as to reliability of some of the methods now in use, with active research to improve them.

Under these circumstances it is believed that a table of single average figures would be unduly tentative and that in most cases a statement of the range within which normal cases and the ultimate average may be expected to fall will be more instructive in the present state of knowledge. This is the meaning of the ranges given in the accompanying table. In any case, one may well be cautious in accepting a supposed average figure which falls outside the range here suggested. For purposes of dietary calculation, one may assume a convenient round figure near the middle of the range; or somewhat lower if there is reason to anticipate serious losses in cooking.

TABLE 65. — VITAMIN VALUES OF FOODS, IN THE TERMS SHOWN AT THE HEADS OF THE RESPECTIVE COLUMNS, PER 100 GRAMS OF EDIBLE PORTION

| FOOD              | ASCORBIC<br>ACID (VI-<br>TAMIN C)<br>MILLI-<br>GRAMS | THIAMIN (VI-<br>TAMIN B <sub>1</sub> )<br>MICROGRAMS | RIBOFLAVIN<br>(VITAMIN G)<br>MICROGRAMS | VITAMIN A VALUE<br>INTERNATIONAL<br>UNITS |
|-------------------|--|--|---|---|
| Almonds           | —  | 120-240 <sup>a</sup>                                 |   | 580 <sup>b</sup>                          |
| Apples            | 5-8  | 20-60  |   | 40-100                                    |
| Apricots, dried   | 2-12   | 60-120   | 240-300                                 | 6,000-15,000                              |
| fresh             | 1-4  | 25-35  | 105                                     | 3,000-8,000                               |
| Artichoke, French | 9  | +  |   | 150-300                                   |
| Asparagus         | 15-40  | 150-180  | ++                                      | 300-700                                   |
| Avocado           | 2-8  | 100-200  | 140                                     | 110                                       |

<sup>a</sup> Estimated range within which the average may be expected to fall.

<sup>b</sup> The printing of a single number in any space in *this* table implies that not enough cases have been found to justify an estimate of the range.

+ or ++ indicates probable relative value.

TABLE 65. — VITAMIN VALUES OF FOODS (*Continued*)

| FOOD                              | ASCORBIC<br>ACID (VI-<br>TAMIN C)<br>MILLI-<br>GRAMS | THIAMIN (VI-<br>TAMIN B <sub>1</sub> )<br>MICROGRAMS | RIBOFLAVIN<br>(VITAMIN G)<br>MICROGRAMS | VITAMIN A VALUE<br>INTERNATIONAL<br>UNITS |
|-----------------------------------|--|--|---|---|
| Bacon                             | —  | (100)  | 75-125                                  | —   |
| Banana                            | 7-8  | 60-100   | 45-80                                   | 160-400                                   |
| Barley, entire                    | —  | 400-500  | +                                       | 71  |
| pearled                           | —  | (180)  | —                                       | —   |
| Beans, dried                      | —  | 400-600  | +                                       | +   |
| Lima, dried                       | —  | 450-600  | 790                                     | +   |
| Lima, fresh                       | 15-35  | 250-350  | 250                                     | +   |
| snap or string                    | 10-20  | 55-95  | 65-150                                  | 600-1800                                  |
| Beef, lean muscle                 | —  | 110-210  | 180-260                                 | 10-50                                     |
| Beet greens                       | 35   | —  | 625                                     | ++  |
| Beets                             | 3-5  | 25-95  | 70-120                                  | <100                                      |
| Blackberries                      | 3  | <25  | —                                       | 80-300                                    |
| Blueberries                       | 4-10   | 45   | 15                                      | 20-80                                     |
| Bran                              | —  | 234-700  | +                                       | 138                                       |
| Bread, Boston brown               | —  | 125-170  | 70                                      | —   |
| rye -                             | —  | 90-190   | +                                       | —   |
| wheat, white                      | —  | 55-85  | 40-100                                  | —   |
| white, raisin                     | —  | (80)   | 45                                      | —   |
| whole wheat                       | —  | 240-400  | 100                                     | —   |
| whole wheat, raisin               | —  | (300)  | (100)                                   | 88  |
| Broccoli                          | 50-130   | 80-100   | 200-500                                 | 3,000-9,000                               |
| Brussels sprouts                  | 13-50  | 171  | +                                       | 300-500                                   |
| Butter                            | —  | —  | —                                       | 3,500-5,000                               |
| Buttermilk                        | 1-2  | 15-50  | 80                                      | —   |
| Cabbage, headed }<br>loose leaf } | 50-90  | 70-140   | 65-135                                  | { 30-80<br>880                            |
| Cantaloupe                        | 26-34  | 50-65  | 75                                      | 400-2,400                                 |
| Carrots                           | 3-5  | 60-140   | 60-120                                  | 2,200-4,000                               |
| Cashew nuts                       | —  | +  | —                                       | —   |
| Cauliflower                       | 48-94  | 130-200  | 150-220                                 | 35-60                                     |
| Celery stalk                      | 6-8  | 20-50  | 30-55                                   | 5-50                                      |
| Chard                             | 10-20  | —  | +                                       | 13,000-27,000*                            |
| Cheese, Cheddar type              | —  | 40-50  | 450-600**                               | 2,000-4,000                               |
| Cottage, skim                     | —  | +  | —                                       | 60-80                                     |
| Cream                             | —  | 15-20  | 100-120                                 | ++  |
| Parmesan                          | —  | 22-30  | 450-600**                               | 1,200-1,500                               |
| Swiss                             | —  | 30   | 450-600**                               | (+)                                       |
| Cherries, canned                  | 6  | —  | —                                       | (+)                                       |
| fresh                             | 8-10   | 51   | —                                       | +   |
| Chestnuts                         | —  | 170-280  | —                                       | —   |
| Chicken                           | —  | 90-380   | 100-200                                 | +   |
| Chocolate                         | —  | 75   | —                                       | —   |
| Clams, long                       | —  | +  | (15)                                    | 10-30                                     |

\* Data for "Greens" used for Chard, Dandelion, Escarole, Kale, Mustard greens, Spinach, and Turnip tops.

\*\* The relatively few data for Cheddar type, Parmesan, and Swiss cheeses are here averaged together, as to riboflavin content.

TABLE 65. — VITAMIN VALUES OF FOODS (*Continued*)

| Food                         | ASCORBIC<br>ACID (VI-<br>TAMIN C)<br>MILLI-<br>GRAMS | THIAMIN (VI-<br>TAMIN B <sub>1</sub> )<br>MICROGRAMS | RIBOFLAVIN<br>(VITAMIN G)<br>MICROGRAMS | VITAMIN A VALUE<br>INTERNATIONAL<br>UNITS |
|------------------------------|--|--|---|---|
| Clams, round                 |  | +  | (15)                                    | 10-30                                     |
| Cocoa, beverage <sup>a</sup> | 1  | 40-60  | (200)                                   | (200)                                     |
| Coconut, dried               | —  | (100-200)  | ++                                      |   |
| fresh                        | —  | 70-100   | ++                                      |   |
| Cod                          | —  | 27-120   | +                                       | 11  |
| Cole slaw                    | 2-20   | 57   | +                                       |   |
| Collards                     | 30-60  | 150-250  | 250                                     | 2,000-6,000                               |
| Corn, canned                 | 6  | (100)  |   |   |
| Corn, maize, dry             | —  | 200-300  | +                                       | *   |
| Corn meal                    | —  | 50-300   | 80                                      | *   |
| Corn, sweet                  | 8-11   | 120-150  | +                                       | *   |
| Cranberries                  | 10-13  |  |   | 10-20                                     |
| Cranberry sauce              | (5-10)   |  |   | (10-20)                                   |
| Cream, thick (40%)           | 1-1.5  | (25-35)  | (120-180)                               | 2,000-2,500                               |
| Cream, thin (18.5%)          | 1-2  | (30-40)  | (150-200)                               | 1,000-1,500                               |
| Cucumbers                    | 2-13   | 90   | 150                                     | 15-50                                     |
| Currants, fresh, red         | 15-20  |  |   |   |
| Dandelion                    | 5-40   | 150-225  | (+)                                     | 13,000-27,000**                           |
| Dates, dried                 | —  | 60-100   |   | 60-300                                    |
| Eggplant                     | 1-9  | 40-100   |   | 20-100                                    |
| Eggs                         | —  | 140-160  | 280-420                                 | 1,000-2,000                               |
| Egg white                    | —  | trace  | 150-300                                 | trace                                     |
| Egg yolk                     | —  | 350-440  | 380-750                                 | 2,500-5,000                               |
| Endive                       | 10-14  | 99   | 235                                     |   |
| Escarole (green)             | 6-10   |  | 100-400                                 | 13,000-27,000**                           |
| Figs, dried                  | —  | 80-180   | 85-125                                  | 50-90                                     |
| Figs, fresh                  | 2  | 80-100   | 82                                      | 60-90                                     |
| Flour, rye                   | —  | 165-220  | 60                                      |   |
| white                        | —  | 60-100   | 40                                      | —   |
| whole wheat                  | —  | (500)  | (100-200)                               |   |
| Gooseberries                 | 25   |  |   |   |
| Grapefruit (or juice)        | 38-41  | 50-100   | 20-100                                  | 21  |
| Grapes                       | 2-3  | 30-60  |   | 20-60                                     |
| Guavas                       | 60-100   | 40-55  | 88                                      | 200                                       |
| Haddock                      | —  | +  | +                                       | 7   |
| Halibut                      | —  | 84-180   | +                                       |   |
| Ham, lean                    | —  | 600-1428   | 200-300                                 |   |
| Hazelnuts                    | —  | 300-500  |   | 440                                       |
| Ice cream <sup>b</sup>       | 1  | (10)   | 55                                      |   |
| Kale                         | 50-100   | 120-190  | 400-600                                 | 13,000-27,000**                           |
| Kidney                       | —  | 400-500  | 1700-2200                               | 500-1000                                  |

<sup>a</sup> Made with milk.<sup>\*</sup> Significant vitamin A values in the yellow, but not in the white varieties. Yellow corn meal reported to have 700-750 International Units per 100 grams.<sup>\*\*</sup> Footnote, p. 569.<sup>b</sup> May vary between the values here given and those of the fruits which it may contain.

TABLE 65. — VITAMIN VALUES OF FOODS (*Continued*)

| FOOD                              | ASCORBIC<br>ACID (VI-<br>TAMIN C)<br>MILLI-<br>GRAMS | THIAMIN (VI-<br>TAMIN B <sub>1</sub> )<br>MICROGRAMS | RIBOFLAVIN<br>(VITAMIN G)<br>MICROGRAMS | VITAMIN A VALUE<br>INTERNATIONAL<br>UNITS |
|-----------------------------------|--|--|---|---|
| Kohlrabi                          | 40-80  | 40-70  |   |   |
| Lamb                              | —  | 200-300  | 280                                     |   |
| Leeks                             | 10-20  | 96   |   |   |
| Lemon (or juice)                  | 52-60  | 30-90  | •                                       |   |
| Lentils                           | —  | 300-600  | 190                                     |   |
| Lettuce, headed }<br>loose leaf } | 6-21   | 50-125   | 100-240                                 | { 70-700<br>700-7,000                     |
| Liver                             | +  | 300-420  | 1,800-2,600                             | 5,000-10,000                              |
| Mangoes                           | +++  | 40-100   | 200-260                                 | 1,000-2,000                               |
| Milk, cows                        | 2.1-2.2  | 40-65  | 195-240                                 | 160-225                                   |
| evaporated                        |  | 50-80  | 330                                     | 300-450                                   |
| dry skim                          |  | 375  | +++                                     |   |
| dry whole                         |  | 315  | 1,300-1,900                             | 1,300-1,800                               |
| malted                            |  | 340  | 500                                     | ++  |
| Mushrooms                         | 3-6  | 100-200  |   | —   |
| Muskmelon                         | 26-34  | 50-65  | 75                                      | 200-2,400                                 |
| Mustard greens                    |  | 138  | 375                                     | 13,000-27,000**                           |
| Mutton                            | —  | 200-300  | (280)                                   |   |
| Nectarines                        |  |  |   | 1,000-2,000                               |
| Oatmeal (dry)                     | —  | 345-770  | 100-200                                 |   |
| Okra                              | 10   | 126  |   | 300-600                                   |
| Olive oil                         | —  | —  | —                                       | 20-30                                     |
| Olives, green                     |  | 8  |   | 388                                       |
| ripe                              |  | (8)  |   | 346                                       |
| Onions                            | 7-11   | 25-100   | 28-62                                   | "negligible"                              |
| Orange (or juice)                 | 52-56  | 75-145   | 28-90                                   | 50-400                                    |
| Oysters                           | 3  | 200-300  |   | 150-300                                   |
| Papayas                           | 33-55  | 15-30  | 83                                      | 2,000-3,000                               |
| Parsnips                          | 14-24  | 120-190  |   |   |
| Peaches, canned                   | 3-5  |  |   |   |
| dried                             |  |  | 150-250                                 | 1,500-6,000                               |
| fresh, white }                    |  |  |   | { 0-100                                   |
| fresh, yellow }                   | 7-10   | 20-70  | 45                                      | { 1,000-2,000                             |
| Peanut(s) roasted                 | —  | 500-600  | 200-500                                 | 360                                       |
| Peanut butter                     | —  |  |   |   |
| Pears, canned                     |  | (30)   |   |   |
| fresh                             | 3-5  | 30-95  | 20-150                                  | 10-15                                     |
| Pea soup                          | 0-1  | 10-15  | 100-200                                 | 300-400                                   |
| Peas, canned                      | 2-10   | 200-300  | 80-200                                  | ++  |
| dried                             |  | 300-620  | 250-380                                 | +   |
| fresh, green                      | 15-25  | 270-495  | 200-250                                 | 1,000-1,300                               |
| Pecans                            | —  | 150-250  |   | 100-200                                   |
| Peppers, green                    | 90-150   | 20-30  |   | ++  |
| Pineapple, canned                 | 10   | 63   | 20-30                                   | 20-30                                     |
| fresh                             | 13-25  | 80-125   | 50-80                                   | 40-60                                     |

\*\* Footnote, p. 569.



TABLE 65. — VITAMIN VALUES OF FOODS (*Continued*)

| Food                       | ASCORBIC<br>ACID (Vi-<br>TAMIN C)<br>MILLI-<br>GRAMS | THIAMIN (Vi-<br>TAMIN B <sub>1</sub> )<br>MICROGRAMS | RIBOFLAVIN<br>(VITAMIN G)<br>MICROGRAMS | VITAMIN A VALUE<br>INTERNATIONAL<br>UNITS |
|----------------------------|--|--|---|---|
| Pineapple juice,<br>canned | 5-10   | 50-100   | 20-30                                   | 40-60                                     |
| Plums                      | 4-7  | 48-200   |   |   |
| Pork, lean                 | —  | 700-1400   | 225-255                                 |   |
| lean, cooked               | —  | 300-900  | (200)                                   |   |
| Potatoes, white            | 7-15   | 95-165   | 40-80                                   | 30-50                                     |
| Prunes, dried              | 0-8  | 175-225  | 50-650                                  | 400-2,400                                 |
| Pumpkin                    | 5  |  | 100                                     | ++  |
| Radishes                   | 12-20  | 50-100   |   | "negligible"                              |
| Raisins                    | —  | 100-200  | 125                                     | 10-100                                    |
| Raspberries                | 8-15   | <25  |   | 130                                       |
| Rhubarb                    | 12-24  | <25  |   | 100                                       |
| Rice, entire               | —  | 240-300  | (+)                                     | 50-100                                    |
| white                      | —  | 30-40  |   | —   |
| Rutabaga, see Turnip       |  |  |   |   |
| Salmon, canned             | —  | +  | 200-250                                 | 20-600                                    |
| Sardines, canned           | —  | 90   |   |   |
| Sauerkraut                 | 0-10   | <30  |   | 25  |
| Shadroe                    | —  | 200-300  |   | 2,000-3,000                               |
| Shrimp                     | —  | 90   |   |   |
| Spinach                    | 15-50  | 95-155   | 300-400                                 | 13,000-27,000**                           |
| Squash, white flesh        | 3  | 42   | 81                                      | 200-400                                   |
| yellow flesh               | 3  | 48   | 81                                      | 2,000-4,000                               |
| Strawberries               | 25-50  | <25  |   | 60-90                                     |
| Sugar                      | —  | —  | —                                       | —   |
| Sweetpotato                | 7-15   | 90-135   | 80-100                                  | 1,500-3,500                               |
| Tangerines                 | 25-50  | 120  |   | 350                                       |
| Tomato (or juice)          | 21-24  | 70-115   | 37-63                                   | 550-1150                                  |
| Turnip(s)                  | 20-30  | 65-95  | 50-100                                  | 10-20                                     |
| Turnip greens              | 20-60  | 138-180  | 750                                     | 13,000-27,000**                           |
| Walnuts                    | —  | 300-600  |   | 100-150                                   |
| Watercress                 | 43-66  | 100-150  | 150-300                                 | 800-3,000                                 |
| Watermelon                 | 6-8  | 30-40  | 30-40                                   | 50-100                                    |
| Wheat, entire              | —  | 500-660  | 100-220                                 | 20-25                                     |
| Wheat germ                 | —  | 2,000-4,000  | 600-800                                 |   |
| Yeast, baker's cake        | —  | 270-700  | 600-3,000                               |   |
| baker's, dried             | —  | 1,000-3,000  | 2,000-4,000                             |   |
| brewer's, dried            | —  | 5,000-8,000  | 2,500-4,700                             |   |

\*\* Footnote, p. 569.

## APPENDIX E

### SIMPLE STATISTICAL TREATMENT OF THE DATA OF NUTRITION INVESTIGATIONS

IN QUANTITATIVE scientific work, whether experimental or observational, it is important not only to bring the data of experiments or observations into comparable numerical form but also to measure, as definitely as may be, the validity of the mean results obtained.

In most modern laboratory courses in physics or in quantitative analysis, the student is taught to compute for a series of measurements or determinations some measure of the precision of the mean result. The *precision measure* thus taught in connection with physics and chemistry may or may not be exactly the same as the classical *probable error* which is so much used in the statistical analysis of observational data (as in economics, sociology, and public health work) and which is equally applicable to the results of laboratory experimentation.

Investigations in the chemistry of food and nutrition, especially since the use of laboratory animals in the study of food values and nutrition problems has become common, often deal with data which involve both the errors of measurement which apply to all laboratory work and also the physiological or individual variability of the experimental animals used — both precision of measurement in the ordinary laboratory sense and the elements of biometrics or of vital statistics as well.

As a matter of fact these two kinds of errors are not so distinct as the ordinary statement of them might seem to imply, the “errors” (variations) in a series of “purely physical” measurements being largely due to physiological variations such as those of eyesight and steadiness of hand in the measurer or measurers. Without elaboration of this consideration, however, it suffices for our present purpose to emphasize the fact that the particular form of precision measure known as probable error (P.E., E., or p.e.) is applicable to the ascertainment of the degree of certainty or

trustworthiness of the result of an investigation whose data are subject to either or both of these kinds of errors or variations. And the computation of the probable error yields incidentally a measure of the variability of the data which (expressed usually in the form of *standard deviation* or *coefficient of variation*) is often of added value in the interpretation of the results.

$$\frac{\text{Standard Deviation}}{\text{Mean}} \times 100 = \text{Coefficient of Variation}$$

Jevons in his *Principles of Science* has given the following concise rules for the computation of probable error,\* and definition of the value thus found:

*Probable Error †*

"The following are the rules for treating the mean result, so as thoroughly to ascertain its trustworthiness.

1. Draw the mean of all the observed results.
2. Find the deviations of each result from the mean.
3. Square each of these deviations.
4. Add together all these squares, which are of course all positive.
5. Divide by one less than the number of observations.†† This gives the *square of the mean error*.
6. Take the square root of the last result; it is the *mean error of a single observation*. (Also called *Standard Deviation*.)

\* Whatever the ambiguity of the term "probable error" the *conception* is one of very great importance which can and should be clearly understood by all students of science.

† Jevons' *Principles of Science*, page 387.

†† In the fifth of the numbered steps above quoted from Jevons, there is a slight difference of usage as to whether the division indicated therein shall be by the number of observations or one less than this number. (That is by a divisor which represents, (1) the number of objects observed, or (2) the number of intervals which one would have if these objects were set up in a row, "in array," for the purpose of observing the extent to which they differ.) This difference should never appreciably affect the conclusions drawn, for if there were one hundred observations the probable error would be affected to the extent of only one hundredth of its numerical value whereas the differences which one would be concerned to interpret would usually be relatively larger. The larger the number of observations the smaller the influence of this deviation of usage, and as the validity of this or any other precision measure is dependent upon the number of observations being large enough to ensure a "fair sample" of the data concerned, it is only when the number of observations is fairly large that it is worth while to compute the probable error. For full discussions of the gathering of data, the judgment of their adequacy for statistical treatment, and related problems see Chaddock's *Principles and Methods of Statistics* (1925).

7. Divide now by the square root of the number of observations, and we get the *mean error of the mean result*. (Standard deviation of the means.)
8. Lastly multiply by the natural constant 0.6745 (or even by  $\frac{2}{3}$ ) and we arrive at the *probable error of the mean result*.

"The probable error is taken by mathematicians to mean the limits within which it is as likely as not that the truth will fall."

The advantage of computing the probable error rather than some other precision measure has been mentioned above. So far as concerns applicability to different types of data, one might equally well use the mean error omitting the final step in the calculation, which consists simply in multiplying this by a constant. The probable error has, however, the added convenience over the mean error (1) of being easily and simply defined in words as the limits within which it is as likely as not that the truth will fall; (2) of easy statement of the numerical likelihood of finding the truth within such wider limits as may be selected, and of the likelihood as to whether or not the difference between the means of two series of observations or measurements is a real or an accidental difference.

*The probable error of the difference between two means is the square root of the sum of the squares of their respective probable errors.\** If the difference between two means were exactly the same as the probable error of this difference, then the chances would be exactly even as to whether the difference were a real or an accidental one; but if the difference were three times its probable error, then (assuming that the sampling was adequate and the data of such a nature as to make the rule applicable) the chances would be better than 20 to 1 that the observed difference was a real one and not attributable to accident or to individual variations.

A probable error is significant only in connection with the mean or the difference to which it applies.

Rietz and Mitchell† summarize as follows the rapidly increas-

\* For a more precise form of statement, see Chaddock, page 239.

† Rietz and Mitchell, *Journal of Biological Chemistry*, Vol. 8, page 305. This paper as a whole argues strongly for the general applicability of Gauss's Law of Error, and the rules derived from it, to the interpretation of the data of nutrition experiments. A relatively skeptical view is presented by Wilson, *Science*, Vol. 58, page 93 (August 10, 1923). Chaddock in his *Principles and Methods of Statistics* gives full and judicial discussions both of the uses and of the possible misuses and limitations of the ordinary statistical methods. See also R. A. Fisher's *Statistical Methods for Research Workers*, 7th Ed., 1938. (Oliver and Boyd.)

ing probability of the validity or reality of a finding or a difference according as the difference of two means is found to be equal to or several times greater than its probable error:

"The exponential equation affords us a means of determining not only certain limits within which the probability is one half that a deviation will fall, *i.e.*, those set by the probable error, but also with what probability we may expect a deviation not to exceed *any* assigned limit. Thus, taking limits that are multiples of the probable error, Gauss's Law of Error enables us to assert that, for variates that follow this law, the chances that another random observation or the mean of any equal random sample will fall within the range  $\pm E$ ,  $\pm 2E$ , etc., are as follows:

|                                |                    |
|--------------------------------|--------------------|
| $\pm E$ the chances are        | even               |
| $\pm 2E$ the chances are       | 4.5 to 1           |
| $\pm 3E$ the chances are       | 21 to 1            |
| $\pm 4E$ the chances are       | 142 to 1           |
| $\pm 5E$ the chances are       | 1,310 to 1         |
| $\pm 6E$ the chances are       | 19,200 to 1        |
| $\pm 7E$ the chances are       | 420,000 to 1       |
| $\pm 8E$ the chances are       | 17,000,000 to 1    |
| $\pm 9E$ the chances are about | 1,000,000,000 to 1 |

"It is improbable, therefore, that the deviation of another random observation will exceed the probable error many times."

*Applicability.* It is presumably not necessary to remind the reader that all these considerations apply only to "chance" or "compensating" errors and to normal individual variations; not to "constant" or "cumulative" errors or errors due to the use of incorrect methods or to biased or inadequate sampling. A more picturesque way of making the distinction is the statement that the Law of Error "applies only to errors and not to mistakes."

This distinction being kept clearly in mind, there still remains the question as to how closely one may expect the chance variations of any given kind of data to approximate the ideal of the perfectly symmetrical frequency distribution for which alone the above rules and ratios hold strictly and precisely true.

For critical discussions of this problem the reader may refer to the papers of Rietz and Mitchell and of Wilson and the textbook of Chaddock already cited as well as to many other books on statistical method written from various points of view.

Of special significance to the student of nutrition is the fact that variations which result from a multiplicity of causes, which plainly include the individual variations encountered in nutrition experiments, have quite regularly been found to approximate closely to the ideal distribution when studied on sufficient numbers of cases; from which it follows that the computation of probable error by the method above cited and its use as here indicated in the interpretation of findings and differences is in full accord with our best scientific knowledge. But unless or until it be actually demonstrated that the particular data at hand do show a symmetrical frequency distribution, the computed probable error should be taken as an indication or approximation rather than as a precisely determined value to be stated dogmatically to several significant places of figures.

It is also unduly dogmatic to suppose that the difference between two means abruptly becomes significant when it reaches a certain ratio to its probable error. A difference 3.5 times greater than its probable error implies (according to Gauss as noted above) chances of about 100 : 1 which scientific workers very commonly consider "statistically significant" for many purposes; but just how good the statistical chances must be to constitute a satisfactory resting point for a particular research is a matter of judgment.



# SUBJECT INDEX

- Ability to resist disease and pre-  
mature aging, 5, 326, 327, 330, 331,  
334, 374-379, 408-409, 422, 517-  
519, 537, 540-546
- Absorption, 81, 95-98, 114, 123
  - of amino acids, 123
  - of protein digestion products, 114,  
123
- Accuracy of our knowledge of calcium  
and phosphorus contents of  
foods, 269-273
- Acetaldehyde, 110
- Acetone, 553
- Acetone "bodies," 113
- Acid(s), acetoacetic, 112
  - amino, 43-50, 62-80, 200-201
  - arachidic, 35
  - arachidonic, 30
  - ascorbic, *see* Vitamin C
  - aspartic, 44, 46, 48, 56, 57
  - benzoic, 553
  - beta-hydroxybutyric, 112, 242, 243
  - bile, 37, 96
  - butyric, 28
  - capric, 28
  - caproic, 28
  - caprylic, 28
  - carbonic, elimination of, 239-241
  - citric, 242, 243, 246
  - decenoic, 30
  - dehydroascorbic, 324
  - diamino-monocarboxylic, 44
  - erucic, 29
  - fatty, 26, 27, 28-30; *see also* Fat(s)
  - "fixed," excretion of, 241-242
  - formic, 243
  - glutamic (glutaminic), 44, 46, 48,  
56, 57, 77
  - glycocholic, 96
  - heterocyclic amino, 44-45
  - hippuric, 242, 243, 246, 553
  - hydrochloric, 94, 242
    - excretion of, 242
  - hydroxyglutamic, 44, 46, 48, 56, 57
  - lactic, 107, 116, 200, 242, 243
- Acid(s), lauric, 28
  - linoleic•(linolic), 29-30
  - linolenic, 30
  - malic, 246
  - monoamino-dicarboxylic, 44
  - monoamino-monocarboxylic, 43-44
  - myristic, 28
  - nucleic, 53
  - of gastric juice, 92-95
  - oleic, 29
  - organic, excretion of, 242, 243
    - in acid-base balance, 242-247
    - in foods, 246-247
  - oxalic, 272-273
  - palmitic, 28
  - pantothenic, 393-394, 399
  - pyruvic, 107, 110, 116, 200
  - quinic, 246
  - salicylic, 553
  - stearic, 26, 28, 200
  - sulfuric, excretion of, 242
  - tartaric, 247
  - taurocholic, 96
  - tetradecenoic, 30
  - thymo-nucleic, 53
  - tritico-nucleic, 53
  - uric, 128, 243, 553
- "Acid albumins," 54
- Acid-base balance, 238-250
  - effect of respiration, 240, 241
  - influence of diet, 243-248
- Acid-forming elements in foods, 243-  
245, 247
- "Acid proteins," 54
- Acidosis, 243
- Action, specific dynamic, of the food-  
stuffs, 170, 171-173
- Activation, antirachitic, 431-432
  - of enzyme, 82
- Activities, internal, 150-168, 169
  - of the digestive enzymes, 81-86
- Activity, mental, question of influ-  
ence on energy metabolism, 162
- muscular, 180-186
- Adequate *vs.* optimal nutrition, 8,



- 253, 326, 331, 335-337, 361-362, 377-379, 421-422, 475-478, 490-491, 504-506, 516-519
- Adipose tissue, 472; *see also* Fat
- Adjustments, dietary, and the national food supply, 522-525, 532-533
- Adrenaline (Adrenine), 74-75, 106; *see also* Epinephrine
- Adrenals, 74, 311
- Adulteration of food, 507
- Age of attainment of major opportunity, 549
- Age, as influencing energy metabolism, 163-166; *see also* Growth
- Agreement of energy computed and found, 144-145
- Alanine, 43, 46, 47, 48, 56, 57, 67, 115, 116, 200  
in intermediary metabolism, 115-116, 200
- Albumin(s), 50, 51, 52, 56, 58, 68, 127
- "Albuminates," 54
- Albuminoids, 53, 57
- Alcohol, cetyl, 24  
myricil, 24
- Alcohol-soluble proteins, 53, 57
- Aldol condensation, 110
- Alfalfa, 566
- Algae, 374
- "Alkali albumins," 54
- Alkali, excess, removal from body, 242
- "Alkali proteins," 54
- Alkaline reserve, 239
- Alkalinity, *see* Hydrogen-ion activity
- Allergy, 507
- Alligator-pear, *see* Avocado
- Almonds, 131, 270, 271, 272, 555, 562, 568
- Alpha-amino acids, *see* Amino acids
- Alpha-amylase, 19
- Alpha-carotene, 402, 403
- Alpha-tocopherol, 447
- Aluminum, 220, 228  
in earth's composition, 220
- Amandin, 52, 56
- Amino acid(s), 43-50, 55-58, 62-80, 86-90, 114-117, 200-201, 473-474, 490-491  
amphoteric nature of, 51-52  
as antecedents of nutritional catalysts, 71-77  
as precursors of hormones, 71-77
- Amino acid(s), chemical structure, 43-47  
dispensable or indispensable as nutrients, 65-68  
elementary composition of, 46  
formation in metabolism from related substances, 64, 66, 67-68  
from enzyme preparations, 87-89  
indispensable, 65-68  
in protein metabolism, 200-201  
nomenclature of, 46  
nutritionally essential, 65-68  
optical activity of, 46  
percentages of, in proteins, 48, 49, 56, 57  
quantitative study of, in proteins, 55-58  
separation from each other, 55-58  
structure of, 43-47
- Aminolipids, 24
- Ammonia, 116, 117, 118, 200, 241-242  
in acid-base balance, 241-242  
in protein metabolism, 116, 117, 118, 200
- Amphoteric, 51, 52
- Amylases, 17, 18, 81, 82, 83, 84, 86, 88-89, 92
- Amylopectin, 19
- Amylopsin, 17, 82, 86
- Amylose(s), 19
- "Anchorage" factor in rickets, 434
- Anemia(s), 279-287  
different types contrasted, 286-287  
hemorrhagic, 280, 281-282  
hypochromic, 280, 284  
idiopathic, 284  
iron-deficiency type, 280, 282-284  
of pregnancy, 285  
pernicious, 280, 282, 284
- Aneurin, *see* Thiamin
- Anhydrase, carbonic, 240
- Animal feeding experiments, *see* under individual topics
- Animal starch, 20
- Anti-grey hair factor, 394, 399-400
- Antineuritic, *see* Vitamin
- Antiphthalmic, *see* Vitamin A
- Antirachitic, *see* Vitamin D
- Antiscorbutic, *see* Vitamin C
- Apparatus for investigation of various topics, *see* discussion of each
- Appetite, 353, 354, 355, 358, 361
- Apple(s), 131, 245, 270, 271, 292, 337,

- 338, 363, 364, 418, 555, 562, 566, 568
- Applicability of statistical interpretation, 269-271, 480, 573-577
- Apricot(s), 218, 555, 562, 568
- Arachidonic acid, 30
- Arachin, 52
- Area of research between the merely adequate and the optimal in food supply and nutrition, 253, 335-337, 361, 422, 491, 505-506, 536-550
- Arginine, 44, 46, 48, 56, 57, 58, 66, 77
- Ariboflavinosis, 375
- Artichoke(s), 555, 562, 568
- Ascorbic acid, 321-347; *see also* Vitamin C  
references, 342-347
- Ash constituents, *see* Mineral elements
- Asparagus, 270, 271, 316, 337, 419, 555, 562, 566, 568
- Aspartic acid, 44, 46, 48, 56, 57
- Aspects, major, of the chemistry of nutrition, 3-7
- Attitude toward newer knowledge, 522-526
- Atwater-Rosa-Benedict respiration calorimeter, 140-144
- Availability, nutritional, of the calcium of different foods, 272-273
- Avitaminosis, *see* discussion of each vitamin
- Avocado(s), 555, 562, 566, 568
- Bacillus acidophilus*, *see* *Lactobacillus acidophilus*
- "Backward art of spending money," 522-525
- Bacon, 130, 131, 555, 562, 569
- Bacteria, experimental injection of, to test relation of vitamin A to incidence and severity of disease, 409  
expulsion of, from respiratory system, 408  
in the digestive tract, 98, 516
- Balance, acid-base, 238-250  
of intake and output, determinations of, as measures of metabolism and requirements, 132, 137-140, 251-259, 264-265, 298
- Banana(s), 131, 245, 292, 337, 338, 364, 380, 418, 556, 562, 566, 569
- Bark as antiscorbutic, 321
- Barley, 68, 556, 562, 566, 569
- Basal energy metabolism, 150-169;  
*see also* under Energy
- Base-forming elements in foods, 244-247
- Beans, baked, 556  
dried, 130, 131, 232, 245, 270, 271, 292, 350, 363, 556, 562, 566, 569  
Lima, 556, 562, 566, 569  
snap or string, 131, 245, 270, 271, 292, 337, 338, 363, 419, 556, 562, 566, 569
- Bed calorimeter, 145
- Beef, 57, 130, 131, 232, 244, 270, 271, 285, 292, 294, 363, 380, 381, 418, 556, 562, 569
- Beeswax, 28
- Beet(s), 245, 270, 271, 292, 556, 562, 566, 569
- Beet greens, 556, 562, 569
- Beet sugar, *see* Sucrose
- Benedict bed calorimeter, 145
- Benedict respiration apparatus, 134, 135-137
- Benedict-Roth portable respiration apparatus, 151
- Benzoic acid, 553
- Beriberi, 348-351, 359-360, 367-372  
references, 367-372
- Beta-amylase, 19
- Beta-carotene, 402, 403, 405
- Beta-hydroxybutyric acid, 242, 243, 553
- Beta-lactose, 17
- Beta-oxidation theory, 111-112
- Bicarbonate, blood, 239-241  
in regulating acid-base balance, 239-241
- Bicarbonate-carbonic acid system as buffer, 239
- Bile, 96-97
- Bile acids, 37, 96
- Bilirubin, 279
- Bioassays, 328-329, 356-358, 359, 379-380, 412-416, 436-438
- "Biological values" of proteins, 209
- Biotin, 393
- Blackberries, 556, 562, 569
- Blacktongue, 386, 388
- Blood, 54, 225, 238, 278-287, 293-294  
abnormalities of, 280-287

- Blood, color index of, 279  
 formation, 279-287  
 glucose content of, 11  
 hydrogen ion activity of, 238  
 mineral content in relation to rickets, 432-434  
 plasma, hydrogen ion activity, 238  
 Blueberries, 556, 562, 569  
 Bluefish, 556, 562  
 Bodily stores, *see* discussion of each nutrient  
 Body, calcium-poor condition of, 253, 260-264, 433, 474  
 elementary composition of, 219-220  
 fat, influence upon protein metabolism, 193  
 not a heat engine, 123  
 Body-weight, conventionally assumed averages for men and women, 132  
 influenced by fluctuations of water balance, 133  
 Bomb calorimeter, 124  
 Bone(s), 221, 225, 227, 253-254, 260-263, 268, 269, 432-435, 475-476  
 as source of dietary calcium, 269  
 Border cells, 93  
 Boys, basal metabolism of, 163, 165  
 energy allowances for, 186-189, 499-501  
 Bradycardia method of determining thiamin, 358  
 Brain(s), 281, 294  
 Brain and nerves, influence of, on basal metabolism, 161-162  
 Bran, 569  
 Brazil nuts, 68, 562  
 Bread, 131, 208, 244, 363, 418, 556, 562, 566, 569  
 Breadstuffs, 101, 508, 510, 512, 513, 526, 527, 529  
 Broccoli, 131, 380, 418, 556, 562, 569  
 Brothers showing effects of different diets, 261  
 Brussels sprouts, 556, 562, 569  
 Budgets, food, 510-535  
 Buffers, in urine, 241-242  
 of blood, 238-239  
 Build of body as associated with differences of composition and energy metabolism, 156, 157  
 Bulk, 20  
 Butter(fat), 30, 33, 35, 131, 316, 417, 418, 526, 527, 529, 554, 556, 562, 569  
 Buttermilk, 556, 569  
 Butyric acid, 28  
 Cabbage, 130, 131, 270, 271, 272, 292, 316, 325, 337, 338, 340, 363, 364, 380, 556, 562, 566, 569  
 Calavo, *see* Avocado  
 Calciferol, 430, 431  
 Calcification, 474, 476; *see also* Calcium, Growth, Rickets  
 Calcium, 220, 222, 223, 225-226, 227, 432-434, 478, 491, 509, 510, 511, 512, 513, 527, 561-565  
 absorption, effect of vitamin D, 433  
 amounts in diets, 222-223, 226  
 amounts needed in nutrition, 251-264, 266-269, 273-277  
 availability of, as furnished by different foods, 272-273  
 content of infant at birth, 457  
 contents of foods, 562-565  
 desirable level of intake, 491  
 dietary standards for, 255-259  
 distribution in body, 225  
 excretion in fasting, 222  
 in bone, 432-434  
 in earth's composition, 220  
 in growth, 474-476  
 ion concentration in blood, 225-226  
 metabolism, 433-434  
 nutritional availability, 272-273  
 nutritional sources of, in different countries, 268-269  
 oxalate, 272-273  
 percentage of, in body, 220, 262-264  
 in growing body as influenced by food, 260-264  
 phosphorus, ratios, 265-266  
 quantitative need for, in nutrition, 251-264, 267-269  
 relation to magnesium, 225  
 relation to sodium and potassium, 225  
 retention in growth and development, 255-264  
 sources of in the food supplies of different parts of the world, 268-269  
 "Calcium rigor," 225  
 Calorie(s), 123-192, 465, 494-500, 509, 510, 527, 553-560  
 and control of body weight, 189-190

- Calorie(s), computed from composition of food, 124-130
  - defined, 125
  - how determined, 124-131
  - per 100 grams of foods (Table), 555-560
  - required per day, 146-147, 157, 180-190, 496-500
- Calorimeter, Atwater bomb, 124-125 bed, 145
- Calorimeter experiments in study of energy requirements in nutrition, 132, 140-149
  - in the study of human nutrition, 140-149
- Calorimetry, 140-147
- Calorimetry, direct, 140-149
  - direct and indirect compared, 144-145
- Cane sugar, *see* Sucrose
- Cantaloupe, 245, 337, 338, 364, 418, 556, 563, 566, 569
- Capric acid, 28
- Caproic acid, 28
- Caprylic acid, 28
- Carbhemoglobin, 240
- Carbohydrate(s), 10-23, 31-32, 53, 81, 94, 101, 196-199, 199-202, 555-560
  - analogy to proteins, 47
  - contents of foods, 555-560
  - conversion to amino acids, 200-201
  - conversion to fat, 31-33, 109-111
  - energy value of, 125-131
  - fate in metabolism, 105-109
  - formation from protein, 116
  - fuel value of, 127-128
  - influence on protein metabolism, 196-200
  - intermediary metabolism of, 105-107
  - metabolism of, 105-111
  - protein-sparing action of, 196-202
  - question of its formation from fat in the body, 114
  - radicles in protein molecules, 45
  - storage in the body, 109
- Carbon, 219, 220
  - amount in proteins, 50
  - and nitrogen balance experiments, 137-140
  - balance experiments, 137-140
  - heat of combustion of, 125
- Carbon, in earth's composition, 220
  - percentage of, in amino acids, 46
  - in body, 220
  - in proteins, 49, 50
- Carbonates, *see* Acid-base balance
- Carbonic acid, elimination of, 239-241
- Carbonic anhydrase, 240
- Caries, dental, as influenced by diet, 478-479
- Carotene(s), 402-405, 416, 417
- Carpentry, 184, 185
- Carrot(s), 130, 131, 245, 270, 271, 272, 292, 316, 337, 363, 380, 418, 556, 563, 566, 569
- Casein, 48, 49, 50, 53, 54, 57, 58, 63, 65, 68-69, 98-99, 127, 232, 473
- Caseinogen, 53; *see also* Casein
- Cashew nuts, 131, 556, 563, 569
- Catabolism, *see* Metabolism
- Catalysts, 71-77, 374; *see also* Enzymes, Epinephrine, Glutathione, Insulin, Thyroxine
- Cauliflower, 556, 563, 569
- Celery, 337, 556, 563, 569
- Cell membranes, 36
- Cells, *see* Blood, Body, Protoplasm, Tissues
- Cellulose, 20-21; *see also* Bran
- Cement substance, intercellular, 325-326
- Cephalin, 24, 25, 36, 37
- Cereals, 101; *see also* under name of each
- Cereal grains, proteins of, 68, 69-71
- Cerebrosides, 24, 25, 36, 37
- Cetyl alcohol, 24
- Chances as found statistically, 573-577
- Characteristics, nutritional, of the chief types of food, 507-513, 515-516
- Chard, 556, 563, 569
- Chart(s), for finding body surface, 155 (DuBois') for determining surface area of man from height and weight, 155
  - of basal metabolism of boys and girls, 163, 164
  - of weight curves in vitamin testing, 357, 414, 415
- Cheese, 131, 417, 510, 512, 513, 526, 527, 529, 556, 563, 569

- Chemical composition of foods, *see*  
under name of each  
"Chemical regulation," 170-171  
Chemistry, as the central science, 1  
of substances concerned in nutri-  
tion, *see* discussion of each  
Chenopodiaceae, 272-273  
Cherries, 557, 563, 569  
Chestnuts, 557, 563, 569  
Chewing of food as a factor in nutri-  
tion and health, 173  
Chicken (and fowl), 57, 363, 557, 563,  
569  
Children, basal metabolism of, 160-  
161, 163-166  
calcium requirements of, 256-264  
calcium retention in, 255-260, 263-  
264, 474-476  
development of, as influenced by  
food, 477-479  
dietary standards for, 211-212  
energy allowances for, 500  
energy metabolism as affected by  
various activities, 186-189  
energy requirements of, 500  
iron requirements of, 290-291  
phosphorus requirements of, 265  
vitamin needs of, *see* discussions of  
the different vitamins  
Chinese, calcium supply of, 269  
Chives, 557  
Chloride, 223-225, 240  
excretion of, 222, 224  
metabolism of, 223-225  
shift, 240  
Chlorine, 220, 221, 222, 223-225, 562-  
565  
contents of foods, 562-565  
excretion in fasting, 221-222, 224-  
225  
in earth's composition, 220  
percentage in body, 220  
Chlorophyll, 10  
Chlorosis, 283-284  
Chocolate, 557, 563, 569  
Cholesterol, 24, 431  
activated 7-dehydro-, 430-431  
Choline, 395-396, 400-401  
Chromatin, 228  
Chylomicrons, 111  
Chyme, 93, 94-96  
Chymotrypsin, 86  
Cilia, 408  
Citric acid, 242, 243, 246  
Citrin, 391, 393, 398  
Citron, 245  
Citrus (fruits), 338, 364, 515, 526, 527,  
529, 531; *see also* Grapefruit,  
Lemons, Oranges  
Clams, 557, 563, 569, 570  
Classification, of amino acids, 43-46,  
65-68  
of anemias, 279-287  
of carbohydrates, 10-21  
of enzymes, 81-82  
of fatty acids, 28-30  
of lipids, 24  
of proteins, 52-54  
Climate in relation to energy metabo-  
lism, 168, 169  
Clothing, *see* Regulation of body  
temperature  
Clotting, 81, 225, 449-450, 453-455  
Coagulated proteins, 54  
Cobalt, 219, 220  
Coccarboxylase, 354  
Cocoa, 557, 563, 570  
Coconut, 56, 557, 563, 570  
globulin, 56  
oil, 35  
Codfish, 130, 131, 316, 557, 563, 570  
Codliver oil, 316, 420, 553  
Coefficient of digestibility of food, 99-  
101  
Coefficient of variation, 574  
Coefficients of variation of calcium,  
phosphorus, and protein require-  
ments, 267  
Coenzymes, 378-379, 385  
Collagen, 53, 553  
Collards, 272, 388, 557, 563, 570  
Colloids, 51  
Color index, 279-280, 281, 282  
Combustion, heat of, *see* Energy value  
Comparison, of calcium, phosphorus,  
and protein requirements, 267  
of energy computed and observed  
(Armsby's), 144-145  
Compensating *vs.* cumulative errors,  
576  
Composition, elementary, as related  
to energy value in foods, 125-127  
of amino acids, 46  
of earth's crust, oceans, atmos-  
phere, 219-220  
of foods, *see* under name of each

- Composition, of human body, 219-220
  - of proteins, 49, 50
- Composition of body as a factor in basal metabolism, 156, 157, 169
- Composition, proximate, of foods, 129-131, 555-560
- Compound lipids, 24
- Compounds compared as to elementary composition and heat of combustion, 127
- Comprehensiveness of certain methods of research, 538-548
- Computation of body surface from weight, 153-161
- Computation of energy value (calories) of food, 124-131
  - of probable error, 574-575
- Conarachin, 52
- Concentration of calcium and phosphate ions in blood, 432-434
- Conch, 316, 563
- Condensation, aldol, 110
- Conduction of heat in regulation of body temperature, 170
- Conjugated proteins, 53-54
- Conjunctivitis, 407, 408
- Constants, dissociation, of organic acids, 243
- Consumption of food as influenced by consumers' purchasing power and knowledge of nutritive values, 489-550
- Consumption, better levels of, 523-535
- Control of body weight, 189-190
- Control of internal environment, 504-506, 547, 548, 550
- Cookery as means of making bone constituents nutritionally available, 269
- Copper, 220, 228, 278-279, 280, 281, 282, 283, 287, 293, 294, 295, 566-567
  - contents of foods, 293, 566-567
- Corn (maize), 53, 56, 57, 68, 208, 209, 557, 563, 566, 570
  - flakes, 131, 557
  - meal, 553, 557, 563, 570
  - oil, 35, 553
  - plant as sole source of ration, 460
  - sugar, *see* Glucose
  - sweet, 270, 271, 337, 557, 563, 570
- Corpuscles, 281; *see also* Blood
- Cost of food, 512-535
- Cottonseed (flour, meal, oil), 35, 553
- Course of food through the digestive tract, 91-98
- Cousins showing effects of different diets, 261
- Cover cells, 93
- Cow as gatherer and preparer of vitamin A values for human nutrition, 417
- Crabmeat, 316
- Crackers, 232, 557
- Cranberries, 316, 557, 563, 570
- Cream, 365, 417, 557, 563, 570
- Creatine, 118
- Creatinine, 118-119, 127, 128, 202
- Cress, 557; *see also* Watercress
- Cretinism, 310
- Crisco, 35
- Critique of animal experimentation, 546-548
- Crops as distinguished from by-products, 533
- Cryptoxanthin, 402, 404
- Crystallization of enzymes, 82, 87-90
- Cucumber, 557, 563, 570
- Cumulative *vs.* compensating errors, 576
- Currants (or juice), 557, 563, 570
- C.V., *see* Coefficient of variation
- Cyclic forms of glucose, 12-14
- Cysteine, 72-73
- Cystine, 44, 46, 48, 56, 57, 58, 64-65, 72, 77, 396
- Cytochrome, 228
- Dandelion(s), 557, 563, 570
- Dasheen(s), 557
- Data, statistical treatment of, 573-577
- Dates, 245, 270, 271, 557, 563, 570
- Deamination, 115-116, 117, 200
  - hydrolytic, 116
  - oxidative, 116
  - simple, 116
- Deathrates as influenced by nutrition, 542-543
- Decenoic acid, 30
- Defence powers of the body as influenced by nutrition, 326, 327, 330, 331, 334, 374-379, 408, 409, 422, 542
- Deficiency diseases, *see* under individual vitamins

- Definiteness of knowledge of calcium and phosphorus contents of certain foods, 269-271  
of vitamin C values of certain foods, 337
- Definitions, introductory, 7-9
- Dehydroascorbic acid, 324
- Dehydrogenase(s), 81
- Demonstration and measurement of vitamin A values of foods, 412-416
- Dental caries, *see* Caries
- Depletion as preliminary to feeding tests for vitamin values, 414
- Deposition of calcium phosphate, *see* Calcium retention, Vitamin D
- Derivation of physiological fuel values of carbohydrates, fats, proteins, and food commodities, 127-131
- Derived lipids, 24
- Derived proteins, 54
- Dermatitis, 392
- Designations, stereochemical, of amino acids, 46
- Destruction of vitamin C, 323, 324, 325
- Determination of vitamin A values of foods, 412-416
- Deuterium (heavy hydrogen) as indicator in studies of fat metabolism, 34
- Development, *see* Growth
- Development of skeleton as influenced by calcium content of food, 474-476
- Development, problem of parallelism between mental and physical, 481
- Deviations, standard, of calcium, phosphorus, and protein requirements, 267
- Deviations, statistical treatment of, 573-577
- Dextrin(s), 19, 91
- Dextrose, *see* Glucose
- d*-Fructose, *see* Fructose
- d*-Glucose, *see* Glucose
- Diabetes, 116
- Diabetic sugar, *see* Glucose
- Diagram of intermediary metabolism, 200
- Dialysis of circulating blood (vivo-diffusion), reference, 119
- Diamino-monocarboxylic acids, 44;  
*see also* under name of each
- Diaminomonophospholipins, 36
- Diastase, 87
- Dienol, 12
- Diet, 168, 189-190, 206-209, 222-223, 243-246, 261-264, 282-287, 331, 365-367, 376, 387-388, 477-479, 489-548  
as factor in antirachitic potency of human milk, 436  
coefficient of digestibility, 101  
influence of on acid-base balance, 243-248  
influence on basal metabolism, 168  
influence on protein requirement, 196-202, 206-209  
in relation to anemia, 282-287  
in relation to food economics, 506-535  
in relation to growth, *see* Growth  
in relation to lactation, *see* Lactation  
in relation to length of life, 516-521, 536-546, 549-550  
in relation to pellagra, 385-388  
in relation to reproduction, 456-461  
*See also* under the individual factors
- Dietaries, 222-223, 489-503, 509-514, 526-534  
food values of American, 509-514
- Dietary standards, 489-503
- Dietary studies, 132-134
- Dieting for control of body weight, 189-190
- Diets A and B compared, 517-519, 542-546
- Differences, statistical interpretation of, 573-577
- Digestibility of foods, 99-101
- Digestion, 81-101  
as facilitating rearrangement of "building stones," 114  
definition of, 7-8  
gastric, 91-95  
intestinal, 95-98  
salivary, 86, 91-93; *see also* Amylase
- Digestive juices, 221
- Dihexoses, 14
- Dihydroxyacetone, 107
- Dipeptides, 47, 54

- Diphosphothiamin (cocarboxylase, thiamin pyrophosphate), 354
- Disaccharides, 14
- Discrimination in the use of dietary standards, 489-491
- Dispersions, 51
- Dissociation constants of organic acids, 243
- Distribution of absorbed amino acids to the tissues, 114
- Distribution of calories, 131, 211-212, 493, 499-501, 555-560
- Distribution of cost and nutrients in American dietaries, 510, 527
- Distribution of expenditure for food, 512-535
- Distribution of mineral elements in foods, 269-271, 291-296, 315-317, 561-567
- Distribution of vitamin values in foods, 337-341, 362-367, 376, 380-381, 387-388, 416-420, 440-441, 568-572
- Distribution-standards for children's dietaries, 500, 501
- Division of science into sciences, 1
- Dreyer prediction-formulas for basal metabolism, 159, 160, 161
- DuBois formula and chart, 153-156
- DuBois respiration apparatus, 136, 144
- DuBois respiration calorimeter, 141, 144
- Duck, 557
- Duclaux terminology of enzymes, 82
- Dysadaptation, 405-406
  
- E., *see* Probable error
- Earth, elementary composition of crust, oceans, atmosphere, 219-220
- Economic prominence of food problems, 2, 506-515, 522-535
- Economics of food, 512-535
- Economy in use of food, 506-515, 522-535
- Edestin, 50, 52, 56, 58, 68, 69, 127, 232, 473
- Efficiency, as influenced by nutrition, 536-538, 541, 542, 548-550
- Efficiency, mechanical, of muscular work, 181-184
- Efficiency of fat as fuel for muscular work, 111, 123
  
- Egg albumin, 50, 51, 52, 56, 232  
molecular weight of, 51
- Egg white, 363, 380, 557, 563, 570
- Egg yolk, 363, 380, 440, 557, 563, 570  
vitamin D in, 440
- Eggplant, 557, 563, 570
- Eggs, 52, 53, 54, 56, 57, 68, 129, 131, 232, 244, 269, 270, 271, 285, 290-291, 292, 294-295, 304, 340, 363, 365, 376, 380, 381, 402, 417, 418, 500, 501, 508, 510, 512, 515, 516, 526, 527, 529, 531, 557, 563, 566, 570
- Electrolytes, 221
- Elementary composition of body, 220  
of earth's crust, oceans, atmosphere, 219-220  
of foods as related to heats of combustion, 125-127  
*See also* names of the elements
- Elimination of carbonic acid, 239  
of fixed acids, 241-242, 246, 248  
of sodium, 224
- Emptying of stomach, 93
- Enamel of teeth, 228
- End-products of protein metabolism, 117-119
- Endive, 557, 563, 570
- Endocrinology, 456
- Energy aspect of food values and nutrition, 3, 123-192, 471-473, 490, 494-500, 509, 510, 511, 513, 527, 553-554, 555-560
- basal metabolism, 150-169  
influence of age and growth, 163-166  
of brain and nerves, 161-162  
of climate, 168  
of foods, 168  
of habits of exercise, 168-169  
of internal activities, 150-152  
of internal secretions, 162-163  
of mental work, 162  
of pregnancy and lactation, 167  
of race, 167-168  
of season, 168  
of sex, 166  
of size, shape, and composition of body, 152-161  
of sleep, 169  
normal standards for, 157-161  
Aub and DuBois, 157-158



- Energy aspect of food values and nutrition, basal metabolism, normal standards for, Dreyer, 159-160**  
Harris and Benedict, 158-160  
Sage Normal Standards, 158  
computed and observed compared, 144-145  
expenditure during muscular labor, 180-186  
metabolism, 132-186  
determination of, 132-151  
by carbon-nitrogen balance, 137-140  
by dietary studies, 132-134  
by direct calorimetry, 140-147  
by respiration experiments, 134-137  
comparison of results of respiration experiments and direct calorimetry, 144  
influence of age and growth, 163-166  
of brain and nerves, 161-162  
of food, 168, 171-173  
of habits of exercise, 168-169  
of internal secretions, 162-163  
of mental work, 162  
of muscular work, 180-186  
of pregnancy and lactation, 167, 186  
of race, 167-168  
of sex, 166  
requirement, 123-192, 471-473, 496-500  
for growth, 471-473  
methods of study, 123-151  
Energy values of foods, 124-131, 555-560  
of food constituents, 124-129  
Enhancement of growth and of adult vitality by nutrition, 260, 377, 422, 470, 516-519, 536-550  
"Enough food," 494-503  
Enterokinase, 97  
Environment, as modifying the hereditary pattern, 470  
control of, in the regulation of body temperature, 169-171  
Environment, internal, 504-506, 547, 548, 550  
Enzyme-coenzyme systems in tissue respiratory metabolism, 378-379  
Enzymes, 13, 14, 16, 17, 19, 81-101  
Enzymes, activities, 84-86  
amylolytic, 81  
chemical nature of, 86-90  
classification, 81-82  
coagulating, 81  
digestive, 81, 84-86, 97  
fat-splitting, 81  
hydrolytic, 81-82  
terminology of, 82  
intestinal, 86, 97  
isoelectric point of, 89  
lipolytic, 81  
oxidizing, 81  
pancreatic, 82, 84, 85, 86, 96  
properties of, 81-91  
protein-splitting, 81  
proteolytic, 81  
reducing, 81  
starch-splitting, 81  
sugar-splitting, 81  
Epinephrine, 74-75, 106, 311  
influence on basal metabolism, 74-75  
Epithelial tissues as affected by shortage of vitamin A, 408-409  
Eradication of beriberi, 348-351  
Erepsin, 86  
Ergometer for study of mechanical efficiency of muscular work, 182-183  
Ergosterol, 429, 431  
activated, 430, 431  
irradiated, 430, 431  
products of irradiation, 431-432  
Ergothioneine, *see* Thioneine  
Error, mean, 574-575  
Error, probable, 573-577  
Errors as distinguished from mistakes, 576  
Errors, compensating *vs.* cumulative, 576  
Errors, statistical treatment of, 573-577  
Erucic acid, 29  
Erythrocytes, 278-287  
formation of, 279-280  
Escarole, 563, 570  
Ether extract, 27  
Etiolated seedlings, 416  
Excelsin, 52, 56, 68  
Excreta, 554  
Excretion of metabolized calcium and phosphorus, 252

- Excretion of mineral salts, 221-222
  - of organic acids, 242-243
- Exercise, 180-186, 186-189, 209-211, 238
- "Exercise of the intelligence" in the control of body weight, 190
- Exophthalmic goiter, *see* Goiter
- Expenditure for foods, 509-515, 522-535
- Expenditure of energy at different occupations, 180-189
- Explanations, introductory, 1-9
- Extension of the prime of life, 377, 422, 476, 517-519, 546-548, 549-550
- Extrinsic factor, 284, 285
- Eye(s), 374, 375
  - symptoms in riboflavin deficiency, 376
- Factor, extrinsic, 284, 285
  - intrinsic, 284, 285
- "Factor 2," 394
- Factor W, 394, 400
- Factors, affecting basal metabolism, summary, 169
  - as yet unmeasured, 492
  - for calorific values of various materials when burned in oxy-calorimeter, 553-554
  - for energy values of foodstuffs, 127-131
  - for fuel value of food, 128
- Farina, 557, 563
- Fasting, 193-194, 221-222
  - energy metabolism, 171-172
  - excretion of mineral elements, 222
- Fat(s), 24-38, 81, 94, 95, 96, 99-100, 101, 127-129, 196-201, 339, 364, 395-396, 497, 500, 501, 553, 554, 555-560
  - absorption of, 34-35, 96, 111
  - as fuel for muscular work, 111, 127-129
  - average elementary composition, 138
  - body, as species characteristic, 33
    - effect of food fat on, 33-35
  - classification of, 24, 36
  - commercial, 245, 508, 510, 512, 526, 527, 529
  - composition, 31, 33
  - contents of foods, 555-560
  - elementary composition of, 33, 127, 138
  - energy value of, 125-131
  - formation from carbohydrate, 31-33, 109-111
    - from protein, 117
  - hardened (hydrogenated), 29
  - heat of combustion of, 127
  - human, 127
  - influence of, on protein metabolism, 194, 197-201
  - metabolism of, 37, 111-113, 395-396
    - beta-oxidation theory, 111-113
  - milk, 33
  - of blood, 111
  - problem of formation from protein in the body, 117
  - protein-sparing action of, 196-201
  - respiratory quotient, 108
  - re-synthesis in intestinal wall, 34-35
  - storage ("depot"), 36, 37
  - storage in the body, 113
  - subcutaneous, as a factor in the regulation of body temperature, 170
  - synthetic, 27
- Fate of organic foodstuffs in metabolism, 105-122
- Fatigue, as a factor in the efficiency of muscular work, 183-184
  - as affecting efficiency of energy metabolism, 183-184
- Fatness, 156, 157, 169, 189-190
- Fat-soluble vitamins, 402-455; *see also* Vitamins A, D, E, K
- Fatty acids, 24, 27, 28-30, 33, 34; *see also* Fat(s)
  - catabolism of, 111-113
  - classification of, 28-30
  - containing deuterium (heavy hydrogen), 34
  - general properties of, 28-30
  - metabolism of, 110-113, 114, 117
  - nutritionally essential, 30
  - synthetic, 27
  - unsaturated, 29-30
- Fatty oils, 26; *see also* Fats, Olive oil, etc.
- Feces, 98, 99-100, 554
- Feeding experiments, *see* individual topics

Ferments, *see* Enzymes

Fetus, *see* Pregnancy, Reproduction

Fever as influencing metabolism, 163

Fibrin, 54

Figs, 557, 563, 570

Filtrate factor, 394, 399

Fish, 57, 563; *see also* name of each  
liver oils, 402, 420, 429, 430, 431-432, 441

Fixed acids, excretion of, 241-242

Fixed oils, 26

Flavin, *see* Riboflavin

Flesh foods, *see* Meat(s) and name of each

Flexibility of the body's internal environment, 504-506

Flora, intestinal, 98

Flounder, 316, 563

Flour, 363, 364, 365, 366, 367, 557, 563, 566, 570  
fortification of, 367  
wheat, 244, 560

Fluctuations of body weight, 133

Fluorine, 228

Food(s), acid-base balance of, 243-248

adulteration, 2, 507

and control of body weight, 189-190

allowances for healthy children, 499-501

antineuritic values of, *see* Thiamin  
antiophthalmic values of, *see* Vitamin A

antiscorbutic values of, *see* Vitamin C

as fuel, 123-145

as sources of vitamins (Table), 568-572

best use of, 506-535

calcium contents of, 269-273

choice of, 504-535

composition of, 555-572

computation of calories (energy or fuel value), 124-131

economics, 506-535

energy (fuel) values, 123-131, 555-560

for gain or loss of body weight, 189-190

industries, 2

influence of intake upon energy metabolism, 171-173

Food(s), influence of, on acid-base balance, 243-248

inspection, 2

iodine contents of, 315-317

mass in digestive tract, 92-98

mineral content of, 561-567

nutritional characteristics of, 507-513; *see also* names of individual foods

nutritional efficiency of the protein contained in, 206-209

passage through digestive tract, 91-98

phosphorus contents of, 269-273

"protective," 515-519

requirements, *see* individual nutrients or factors

supply, 506-535

Foodstuffs, specific dynamic action, 170, 171-173

specific dynamic action upon energy metabolism, 171-173

*See also* name of each

Formation of fat from carbohydrate, 109-111

Formation of vitamin A from its precursors, 402-405

Formic acid, 243

Formula(s),

for computing surface area by height and weight, 154-156

for estimating body surface from weight, 153-156

structural, for alpha-carotene, 403

for amino acids, 43-45, 46

for beta-carotene, 403

for calciferol (vitamin D<sub>2</sub>), 430

for cholesterol, 431

for cryptoxanthin, 404

for 7-dehydrocholesterol (irradiated), 430

for ergosterol, 429

for fatty acids, 28-30

for gamma-carotene, 404

for glutathione, 72

for riboflavin (vitamin G), 373

for thiamin, 354

for thioneine, 231

for thyroxine, 73

for vitamin A, 403

for vitamin B<sub>1</sub> hydrochloride, 354

for vitamin C, 323

- Formula(s),
  - for vitamin D<sub>3</sub> (irradiated 7-dehydrocholesterol), 430
- Fortification of white flour, 367
- Fowl(s), 557, 563
- Fractionation of the nitrogen of proteins, 55-58
- Frequency diagram for weights of growing rats, 480
- Fructose, 11, 12
- Fruit(s) (and their juices), 21, 98, 101, 246-247, 316, 335, 336, 363, 364, 365, 366, 500, 501, 508, 510, 512, 513, 515, 516, 526, 527, 529, 531, 534; *see also* names of individual fruits
  - acid-base balance, 242-248
  - as antiscorbutics, 321, 322; *see also* the names of individual fruits, Tables of vitamin C values, and Vitamin C
  - citrus, 338, 364, 515, 526, 527, 529, 531
  - organic acids in, 242-248
- Fruit sugar, *see* Fructose
- Fuel values of food, 124-131, 508-511, 527, 555-560
- Functions of food defined, 7
- Functions of stomach summarized, 95
- Fundus, 92-93
- Furanose, 13
- Gain or loss of body weight, interpretation of, 133
- Galactans, 14
- Galactolipins, 36; *see also* Cerebrosides
- Galactose, 13, 45
- Galactosides, 14, 17; *see also* Cerebrosides
- Gamma, 359
- Gamma-carotene, 402, 404
- Gastric digestion, 91-95
- Gastric juice, 92-95; *see also* Pepsin
- Gelatin, 48, 49, 53, 57, 68, 127
- Gestation, *see* Reproduction
- Girls, basal metabolism of, 164, 165
  - energy allowances for, 186-189, 499-501
- Gliadin, 48, 49, 50, 53, 57, 58, 63, 68-69, 127, 232, 473, 553
- Globin, 53
- Globulins, 52, 56
- Glucose, 11, 12, 127, 200, 553
  - Glucose, concentration in the blood, 106
    - heat of combustion of, 127
    - metabolism of, 11, 105-109
    - occurrence in nature, 11
    - produced from amino acids, 116-117
    - relation to fat metabolism, 200
    - relation to other carbohydrates, 11-14, 15, 16, 17, 18, 19, 20
    - relation to protein metabolism, 116-117, 200
  - Glutamic (glutaminic) acid, 44, 46, 48, 56, 57, 72
  - Glutaminic acid, *see* Glutamic acid
  - Glutathione, 72-73, 230
  - Glutelins, 52, 56, 68
    - (maize), 68
  - Glutenin, 53, 56, 68
  - Glutin meal, 554
  - Glyceric aldehyde, 107, 200
  - Glycerides, 27-31
  - Glycerol, 200
  - Glycine, 43, 46, 47, 48, 49, 56, 57, 67, 72
  - Glycinin, 56, 68
  - Glycocholic acid, 96
  - Glycocoll, *see* Glycine
  - Glycogen, 20, 106, 109, 110, 127, 196
  - Glycolipids, 24, 36; *see also* Cerebrosides
  - Glycoproteins, 53
  - Glycosuria, 116
  - Glycylglycine, 47
  - Goal, optimal nutrition as, 253, 481, 504-519, 522-550
  - Goiter, 307-315
    - adenomatous, 314
    - as influencing metabolism, 162-163
    - prevention, 310-315
  - Gooseberries, 557, 563, 570
  - Goosefoot Family, 272-273
  - Grain products, 508, 510, 511, 512, 526, 527, 529
  - Grains, 316, 339, 363, 364, 380, 500, 501
    - see also* Breadstuffs, Cereals, and under name of each
  - Grapefruit or juice, 131, 337, 338, 557, 558, 564, 570
  - Grape(s), 558, 564, 566, 570
  - Grape juice, 558
  - Grape sugar, *see* Glucose
  - Grass juice factor, 394, 400

- Greenness of plant tissue and its vitamin A value, 402, 416-419
- Greens, 272-273, 338, 363, 364, 376, 381, 402, 417, 418; *see also* Leaves, Vegetables, and name of each
- Grouping of foods for dietary planning, 526-529
- Growth, 470-488
- as influenced by energy supply, 471-473
  - as influenced by food habits of children, 477-479, 536-542
  - as influenced by mineral elements, *see* name of each element
  - as influenced by supply of proteins, 473-474
  - as influenced by supply of vitamins, *see* each vitamin
  - calcium requirements for, 255-260, 475-476
  - energy requirement of, 499-501
  - experiments as a means of research, 479-481
  - experiments investigating proteins and their amino acids, 63-71, 77-80
  - experiments with, as a means of research, 479-481
  - frequency diagrams of, 480
  - linear, as influenced by vitamin D, 435-436
  - problem of ideal rate, 481
  - references, 481-488
- Guavas, 558, 570
- Habits of exercise, influence on basal metabolism, 168-169
- Haddock, 558, 564, 566, 570
- Halibut, 57, 558, 564, 566, 570
- Halibut-liver oil, 420
- Ham, 564, 570
- Hardened (hydrogenated) fats, 29
- Harris and Benedict formula for normal basal metabolism, 158
- Hay as source of vitamin A through the cow, 417
- Hazelnuts, 558, 564, 570
- Health as a positive quality of life, 536-550
- Heart, 225-226, 294, 564
- regulatory action of ions, 225
- Heats of combustion of the foodstuffs, 124-128
- Heat production, *see* Metabolism
- Heating, effect of,
  - on vitamin C, 340-341
- Height-weight formulas for body surface, 154-156
- Hematopoiesis, 282, 284
- Hematopoietic, 284, 285
- Hemeralopia, 405-406
- Hemicelluloses, 20-21
- Hemoglobin, 50, 53, 228, 238, 239, 240, 278-286
  - as buffer, 238, 239, 240
  - concentration in blood, 238
  - in oxygen transfer, 240
  - in transfer of carbon dioxide, 240-241
  - molecular weight of, 50
- Hen as gatherer and preparer of vitamin A values for human nutrition, 417
- Hepatoflavin, *see* Riboflavin
- Heredity, 470-471
- Herring, 558
- Heterocyclic amino acids, 44-45
- Hexobioses, 14
- Hexosediphosphate, 105
- Hexosephosphate, 105
- Hippuric acid, 242, 243, 246, 535
- Histidine, 44, 46, 47, 48, 56, 57, 58, 66, 72, 77, 231
- Histones, 53
- Holism, 492
- Home-growing of fruits and vegetables, 533-534
- Hominy, 558, 564
- Honey, 558, 564, 566
- Hordein, 53, 57, 68
- Hormones, 37, 71-77; *see also* Catalysts, Epinephrine, Glutathione, Insulin, Secretin, Thyroxine
- Housing, *see* Regulation of body temperature
- Huckleberries, 558, 564; *see also* Blueberries
- Humin (melanin) nitrogen, 58
- Hundred-Calorie portions of foods, 130-131, 555-560
- Hunger, 91
- Hydrochloric acid of gastric juice, 94
- Hydrogen, 219, 220
  - amount in proteins, 50
  - heat of combustion of, 125
  - in earth's composition, 219-220

- Hydrogen, percentages of, in amino acids, 46
  - in body, 219-220
  - in proteins, 49, 50
- Hydrogenation of fat, 29
- Hydrogen-ion activity, 83, 84, 238
  - as affecting stability of vitamin C, 341
  - influence on enzymes, 83-84
  - of blood plasma, 238
  - of tissues, 247
  - of urine, 241-242
- Hydrogen-ion concentration, *see* Hydrogen-ion activity
- Hydrolysis of starch by pancreatic amylase, 84
- Hydroxyglutamic acid, 44, 46, 48, 56, 57
- Hydroxyproline, 45, 46, 48, 56, 57
- Hypothyroidism as influencing metabolism, 163
- Ice cream, 365, 417, 558, 570
- Identification of enzymes, hormones, vitamins, *see* general discussion of each
- Improvement of adequate diet, 377-378, 517-519
- Improvement of health by use of the newer knowledge of nutrition, 517-519, 522-525, 531-532, 536-550
- Improvement of nutrition of the people both an economic and an educational problem, 514
- Incidence or duration of disease as influenced by vitamin A value of food, 409-410
- Indispensability of certain amino acids, 65-68
  - of certain fatty acids, 30
- Infancy, special problem of maintenance of body temperature, 171
- Infant, relative effectiveness of different forms of vitamin D, 431
- Infants, *see* Children
- Influence, of brain and nerves on basal metabolism, 161-162
  - of diet on acid-base balance, 243-248
  - of food upon internal environment, 504-506, 547, 548
- Influence, of muscular work on energy metabolism and food requirement, 180-186
- Inorganic foodstuffs or elements, *see* Mineral elements, and name of each
- Insulin, 75-77, 106
  - crystalline forms of, 75-77
  - molecular weight of, 77
  - protein nature of, 77
- Intake, the concepts of adequate and optimal, 8, 253, 326, 331, 335-337, 361-362, 377-379, 421-422, 475-478, 490-491, 504-506, 516-519
- Interconvertibility of three-carbon compounds, 107, 111, 116, 200
- Intermediary metabolism, 105-122, 200-202, 360
- Internal environment, 326, 331, 332, 377, 421-422, 504-506, 547
- International units of vitamin value, 325, 359, 413, 437
- Interpenetration of chemistry, physics, and physiology, 1
- Interrelations, of amino, hydroxy, and ketonic acids, 107, 116, 123, 200
- Interrelationships, among intermediates of carbohydrate metabolism, 107
  - in the chemistry of nutrition, 5-6
- Intestine, 95-98
- Intrinsic factor, 284, 285
- Inversion, 16
- Invert sugar, 16
- Invertase, *see* Sucrase
- Iodide as preventive of goiter, 310-315
- Iodine, 220, 228, 307-320
  - amount in the body, 312
  - amounts needed in normal nutrition, 312-313
  - requirement in nutrition, 312-315
- Iodine number, 29, 35
- Iodized salt, stabilization of, 315
- Iodothyroglobulin, 311
- Iron, 50, 220, 223, 228, 229, 278, 306, 509, 510, 512, 513, 527, 561-565
  - amount in the body, 278
  - availability of in certain foods, 291, 293-296
  - concentration in the blood as compared with the body as a whole, 278

- Iron, content of the body, 278  
   contents of foods, 561-565  
   distribution of, in the body, 228, 278  
   forms of, used in anemia, 282  
   functions in normal nutrition, 278-279  
   importance in normal nutrition, 278-279  
   in American dietaries, 223  
   in earth's composition, 220  
   in foods, 291-296  
   in growth, 474  
   medicinal, 282, 284  
   normal nutritional function, 278-279  
   percentage in body, 220  
     in various foods, 291-296, 562-565  
   requirement in nutrition, 287-290, 491  
   reserve in the body at birth, 282-283, 474  
   therapeutic use, 281-287  
 Irradiation products, 430-432; *see also* Vitamin D  
 Islands of Langerhans, 106  
 Islet cells, 106  
 Isoelectric, 52  
 Isoelectric point, 89  
 Isoleucine, 43, 46, 66  
 Isomerism in proteins, 50  
  
 Jams and jellies, 558  
 Jevons' rules, 574-575  
 Juice, gastric, 92-95  
   intestinal, 95, 96, 97-98  
   pancreatic, 96  
  
 Kafirin, 53  
 Kale, 131, 270, 271, 272, 292, 364, 380, 388, 415, 418, 558, 564, 566, 570  
 Kelp as source of iodine, 314  
 Kephalin, *see* Cephalin  
 Kerasin, 36  
 Keratomalacia, 407, 408; *see also* Vitamin A  
 Ketogenic-antiketogenic acid and ratio, 112-113  
 Ketosis, 112-113, 243  
 Kidney(s), 281, 294, 363, 380, 564, 570  
   action of, as a factor in acid-base balance, 241  
  
 Knowledge of organic chemistry pre-supposed, 9  
 Kohl-rabi, 558, 564, 570  
 Kryptoxanthin, *see* Cryptoxanthin  
  
 Lactal, 12  
 Lactalbumin, 50, 52, 56, 58, 68, 69, 70, 473  
 Lactase, 16, 81, 86  
 Lactation, 461-462, 465-466  
   as test of nutritive value of diet, 460-461  
   energy requirements for, 186  
   in the human mother, nutritional support of, 465-466  
 Lactic acid, 107, 116, 200, 242, 243, 553  
*Lactobacillus acidophilus*, 17, 19, 98  
 Lactoflavin, *see* Riboflavin  
 Lactone(s), 12  
 Lactose, 14, 16-17, 93, 553  
 Lamb and mutton, 294, 363, 558, 564, 571  
*Laminaria religiosum*, 314  
 Langerhans, islands of, 106  
 Lard, 35  
 Lard substitute, 35  
 Lauric acid, 28  
 Lavoisier as "the father of the science of nutrition," 8-9  
 Law of error, 576  
 "Law of the minimum," 504  
 Leaves, 381, 402, 417; *see also* name of each  
 Leaves and twigs as antiscorbutics, 321  
 Lecithin, 24, 25, 36, 37  
 Lecithoproteins, 54  
 Leeks, 272, 571  
 Legumelin, 52, 56  
 Legumes, 101, 316, 339, 363, 364, 501  
   copper content, 293; *see also* under name of each  
   dry, 526, 527, 529  
   *See also* Beans, Peas, Lentils  
 Legumin, 50, 52, 56, 127, 232  
 Lemon (or juice), 245, 322, 337, 564, 571  
 Length of life, as bearing on cultural development, 537, 549-550  
   as influenced by nutrition, 377, 422, 517-519, 537, 539-546  
 Lentils, 558, 564, 571

- Lettuce, 131, 272, 316, 337, 363, 380, 558, 564, 566, 571
- Leucine, 43, 46, 48, 56, 57, 66, 77
- Leucosin, 50, 52, 56, 232
- Level of expenditure for food as influencing the diet, 513-515
- Level of protein intake as influencing proportions of end products, 118-119
- Level of protein metabolism as influencing its end products, 118-119
- Levels of nutritional intake, problem of the optimal, 8, 253, 326, 331, 335-337, 361-362, 377-379, 421-422, 475-478, 490-491, 504-506, 516-519
- Levulose, *see* Fructose
- Life expectation, 517-519, 539-546; *see also* Health, Length of life
- Light-adaptation (dark adaptation), 406
- Limes, 558
- Limitations of dietary standards, 489-491, 496
- "Line test" measure of vitamin D, 436-438
- Linkage of amino acids in peptides and proteins, 47
- Linoleic acid, 29
- Linolenic acid, 30
- Linolic acid, *see* Linoleic acid
- Linseed meal, 209, 554
  - oil, 30
- Lipase(s), 81, 82, 86, 88
- Lipids, 24-38
  - as body constituents, 35-37
  - classification of, 24
- Lipins, *see* Lipids
- Lipoids, 24-38
- Lipolytic enzymes, *see* Lipases
- Lips, cracking of, as symptom of riboflavin deficiency, 376
- Lissauer's formula for surface-weight relationship, 153
- Liver, 280, 281, 282, 285, 293, 294, 363, 380, 419, 558, 564, 566, 571
- l-(+) and l-(-) designations of amino acids, 46
- Local factor in rickets, 434
- Loganberries, 564
- Longevity, *see* Length of life
- Low-calcium rickets, 433
- Low-phosphorus rickets, 432-433
- Lumberman, 184
- Lying, 185
- Lysine, 44, 46, 48, 49, 56, 57, 58, 63-64, 66, 72, 77, 473-474
- Macaroni, 558, 564
- Mackerel, 564, 567
- Macrocytis pyrifera*, 314
- Magnesium, 220, 222, 223, 225, 226, 562-565
  - amounts in dietaries, 223
  - contents of foods, 562-565
  - distribution in body, 225
  - excretion in fasting, 222
  - in American dietaries, 223
  - in earth's composition, 220
  - percentage in body, 220
  - relation to calcium, 225
- Maintenance, of acid-base balance in body, 238-248
  - of body temperature, 169-171
- Maize (corn), 53, 56, 57, 68, 208, 209, 557, 563, 566, 570
  - glutelin, 52-53, 56
  - oil, 35
- Major aspects of nutrition enumerated, 1-9
- Malic acid, 246
- Malt diastase, 19
- Malt sugar, *see* Maltose
- Maltase, 81, 86
- Maltose, 14, 17
- Man, nitrogen output of, compared with other species, 202
- Manganese, 220, 229-230, 283, 566-567
  - contents of foods, 566-567
  - distribution in body, 229-230
  - in earth's composition, 220
  - in milk, 229
  - in plants, 229
  - percentage in body, 220
- Mangoes, 571
- Mannose, 14, 45
- Maple sirup, 564
- Marrow, 279
- Mastication, 91
  - as a factor in nutrition and health, 173
- Maturity as influenced by food, 472
- Mayonnaise, 559
- Meal, corn (maize), 553, 557, 563, 570



- Meat(s), 50, 52, 53, 57, 99, 209, 270, 271, 292, 293-294, 339, 363, 364, 365, 380, 381, 388, 500, 501, 508, 510, 512, 513, 526, 527, 529, 531, 564; *see also* under name of each
- Mechanical efficiency of muscular work, 181-184
- Mechanics of digestion, 92-99
- Mechanism of protein-sparing action, 196-201
- Meeh's formula for surface-weight relationship, 153
- Membranes, 36
- Mental and physical development, problem of relation between, 481
- Mental work, 162
- Metabolism, 395-396  
     as influenced by internal secretions and pathological conditions, 162-163  
     basal, 150-169  
         as influenced by age, 163-166  
         as influenced by race, 167-168  
         as influenced by sex, 166-167  
         by nutritional condition, 168  
     Benedict's summary of chief factors, 169  
     DuBois' estimate of normal limits of variation, 156  
     in elderly people, 166  
     influence of brain and nerves, 161-162  
     influence of climate, 168  
         of food, 168  
         of season, 168  
         of sleep, 169  
     of athletes, of non-athletic men, and of women, compared, 157  
     of boys at different ages, 163, 165  
     of children, 160-161, 163-166  
     of girls at different ages, 164, 165  
     portable apparatus for determination of, 151  
     references, 174-179  
     conditions affecting, 193-202  
     defined, 7-8  
     during fasting, 193-194  
     during sleep, 152  
     energy, as immediately affected by muscular activity, 180-189, 191-192
- Metabolism, energy, as quantitatively related to work performed (mechanical efficiency of muscular work), 181-184  
     references, 174-179, 191-192  
     total per day as affected by muscular activity, 180-189, 191-192  
     while dressing and undressing, 185  
     while knitting, 185  
     while lying still, 185  
     while occupied in different ways, 184, 185, 186  
     while reading aloud, 185  
     while running, 185  
     while sewing, 185  
     while singing, 185  
     while sitting at rest, 185  
     while sleeping, 185  
     while standing, 185  
     while swimming, 185  
     while typewriting, 185  
     fate of foodstuffs in, 105-122  
     mineral, 219-232  
     of carbohydrate, 105-114  
     of chlorides, 223-225  
     of elderly people, 166  
     of energy as influenced by food intake, 171-173  
         in fasting, 172  
         in muscular work, 180-186  
         in refeeding after fasting, 172  
     of individual elements, *see* discussion of each  
     of nucleic acid, 117, 118  
     of protein, 193-202  
     respiratory, 132, 134-137; *see also* Energy
- Metals, *see* name of each
- Metaplasia, 407, 408, 409; *see also* Vitamin A deficiency
- Metaproteins, 54
- Methionine, 44, 46, 64, 66, 396
- Methods, *see* discussions of individual topics
- Methylglyoxal, 107, 116, 200
- Microgram, 359
- Microorganisms, in determination of thiamin, 358
- Milk, 68-71, 98, 100, 131, 208, 209, 211-212, 227, 232, 269, 270, 271, 272, 285, 289, 292, 295, 316, 337,

- 340, 363, 364, 365, 366, 376, 380, 381, 388, 402, 417, 418, 462-465, 477-478, 500, 501, 508, 510, 512, 513, 515, 516, 526, 527, 529, 558, 564, 567, 571
- composition in relation to rate of growth of species, 227
- condensed, 558
- dried, 558, 571
- enrichment with vitamin D, 441
- evaporated, 558, 571
- fat, composition of, 33
  - formation of from carbohydrate, 32
  - influence of food upon, 33
  - vitamin D in, 440-442
- fat contents of successive portions drawn, 464
- human, *see* Lactation
- irradiated, 441
- malted, 571
- manganese in, 229
- nutritional provision for production of, 462-466
- of different species, 227
- proteins of, *see* Casein, Lactalbumin
- sugar, *see* Lactose
- variations in quantity and composition as influenced by nutrition, 462-465
- "Vitamin D milk," 441
- vitamin D value of natural, 440-442
- "Milligrams per cent," 331
- Mineral content of foods, 561-567
- Mineral elements, 219-232, 238-248, 561-567
  - excretion in fasting, 221-222
  - general functions, 221
  - in American dietary, 222-223
  - in earth's composition, 219-220
  - percentage in body, 219-220
  - relation to acid-base balance, 238-248
- See also* under name of each
- Mineral metabolism, 219-232
- Mineral oil as influencing utilization of carotene and vitamin A, 445
- Mistakes *vs.* the errors of legitimate deviation, 576
- Mixed triglycerides, 30-31; *see also* Fats
- Mobilization, *see* Metabolism and discussions of individual nutrients
- Molasses, 558, 564
- Molecular weights of proteins, 50-51
- Monoamino-dicarboxylic acids, 44
- Monoamino-monocarboxylic acids, 43-44
- Monoamino-monophospholipins, 36
- Monosaccharides, 10-14
- Mother, food needs of, in reproduction and lactation, 465-466
- Motility of digestive tract as influenced by thiamin, 354
- Mottled enamel, 228
- Movements of digestive tract, 91-96
- Mucin(s), 53
- Muscle globulin (myosin), 50, 52
  - tension or tone as a factor in basal metabolism, 150, 151
- Muscular exercise (or work), influence on energy metabolism and requirement, 180-186
  - influence on protein requirement, 209-211
- Mushrooms, 558, 564, 571
- Muskmelon, 558, 564, 571; *see also* Cantaloupe
- Mustard greens, 571
- Mutase(s), 82
- Mutton, 564
  - fat (tallow), 34
- Myosin (muscle globulin), 50, 52, 232
- Myricil alcohol, 24
- Myristic acid, 28
- Myxedema, 309
- "Nature and nurture," 470-471
- Nectarines, 571
- Needs, nutritive, *see* Growth, Maintenance, Metabolism, Requirements, Standards, and under each nutrient
- Nervon, 36
- Neuritis, multiple peripheral, 348-351; *see also* Beriberi, Thiamin
- Neutrality, *see* Acid-Base balance
- Nicotinic acid, 378, 385-390
  - chemical structure, 386
  - structural formula, 386
- Night-blindness, 405-406; *see also* Vitamin A deficiency
- Nitrogen, 193-312, 219, 220, 221, 222, 231-232

## SUBJECT INDEX

- Nitrogen, amount in adult body, 219–220**  
 amount in protein, 55, 58  
 balance experiments, 194–196  
 content of infant at birth, 457  
 equilibrium, 208  
   definition, 194  
   at different levels of protein intake, 194–196  
 excretion in fasting, 221–222  
 fate of, in protein metabolism, 117–119  
 in earth's composition, 219–220  
 in protein metabolism, 193–203, 204, 210  
 percentages of, in amino acids, 46  
   in body, 219–220  
   in proteins, 49, 50  
 storage in body, 196  
 storage of, during pregnancy, 457–458  
 to sulfur ratio, in foods, 232  
 to proteins, 231–232  
 “No calories without vitamins,” 190  
**Nomenclature, see Classification**  
**Non-amino nitrogen, 58**  
**Non-fat Calories, as basis for thiamin requirement, 359–360**  
**Norleucine, 43, 46**  
**Nucleic acid, 53**  
**Nucleoproteins, 53**  
**Numbers of polypeptides and proteins, 50**  
**Nut bread, 553**  
**Nutrients, organic, in foods, see Carbohydrate, Fat, Protein, and names of individual vitamins**  
**Nutrition, as the functional aspect of food chemistry, 9**  
   adequate *vs.* optimal, 8, 253, 326, 331, 335–337, 361–362, 377–379, 421–422, 475–478, 490–491, 504–506, 507, 516–519  
   different uses of the word, 8–9  
**Nutrition can be standardized at different levels, 489; see also discussions of optimal intakes of Calcium, Riboflavin, Vitamin A**  
**Nutritionally essential amino acids, 65–68**  
**Nutritionally essential fatty acids, 30**
- Nuts, 316, 510**  
 copper content, 292; *see also* under name of each
- Oatmeal (Oats), 131, 232, 244, 292, 316, 363, 558, 564, 567, 571**  
**Oat-plant as sole source of ration, 459–460**  
**Objective of scientific research, 1**  
**Objective of study of dietary standards, 493**  
**Occupation, as affecting energy requirement, 147, 161–162, 168–169, 180–189, 191–192**  
**Occurrence of elements and substances, see name of each**  
**Oils, salad, 131; see also name of each**  
**Okra, 558, 564, 571**  
**Oleic acid, 29**  
**Olive(s), 245, 558, 564, 571**  
   oil, 553, 571  
**Onions, 245, 558, 564, 567, 571**  
**Optimal defined, and use of the term discussed, 253**  
**Orange (or juice), 131, 245, 292, 322, 331, 332, 337, 363, 364, 380, 418, 558, 564, 567, 571**  
**Organic acids, excretion of, 242, 243**  
   in acid-base balance, 242–247  
   in foods, 131, 246–247  
**Osmotic pressure, 221, 223, 225**  
**Ossein, 553**  
**Osteoporosis, 433**  
**Ovalbumin, see Egg-albumin**  
**Overlapping of chemistry, physics, and physiology, 1**  
**Overweight, 189–190**  
**Ovoflavin, see Riboflavin**  
**Ovovitellin, 50, 53, 57**  
**Oxalate(s), 247, 272–273**  
   and oxalic acid, relation to availability of calcium, 272–273  
**Oxalic acid, 243**  
**Oxidases, 81**  
**Oxidation, 105, 311, 374**  
   catalysts of, 311, 374  
   of carbohydrate in the body, 107, 108  
   of fat in the body, 108, 111–113  
**Oxidation-reduction, 72–73, 278, 385**  
   method for determination of vitamin C, 324  
**Oxidation-reduction (redox) systems, 278, 385**

- Oxy-calorimeter, 553-554
- Oxy-calorimeter of Benedict, 125-126
  - factors for values of various materials when burned in (Table), 553-554
- Oxygen, 219, 220
  - amount in protein, 50
  - consumed, observation of as a measure of energy metabolism, 132, 134-137
  - in earth's composition, 219-220
  - percentages of, in amino acids, 46
  - in body, 219-220
  - in proteins, 49, 50
  - transfer in blood, 239-241
- Oxygen bridge formulas for glucose and other simple sugars, 12-14
- Oxygen carriers, 228
- Oxyhemoglobin, 50, 53
- Oysters, 131, 244, 316, 558, 564, 567, 571
  
- Palmitic acid, 28
- Pancreas, 281
- Pancreatic amylase, 82, 84, 86, 87-88
- Pancreatic juice, 96
- Pantothenic acid, 393-394, 399
- Papayas, 571
- Parietal cells, 93
- Parsley, 564
- Parsnips, 270, 271, 559, 564, 571
- Partition of nitrogen, 58, 117-119, 203
- Passage of food through digestive tract, 91-98
- Pastures as source of vitamin A values, 417
- P.E., *see* Probable error
- Pea soup, 571
- Peaches, 281, 337, 559, 564, 571
- Peanut(s), 131, 363, 388, 559, 564, 571
  - butter, 559, 571
  - oil, 35
- Pears, 245, 337, 559, 564, 567, 571
- Peas, 56, 131, 232, 559, 564, 567, 571
  - dried, 292, 363
  - fresh, 337, 338, 363, 364
  - green, 388, 418
- Pecans, 559, 565, 571
- Pellagra, 385-390
  - as recently defined, 386
  - usually complicated by other nutritional deficiencies, 387-388
- Pellagrins, typical diet of, 387
- Peppers, green, 419, 559, 565, 567, 571
- Pepsin, 82, 83-84, 86, 87, 88, 89
- Pepsinogen, *see* Enzymes, Pepsin
- Peptic digestion, *see* Pepsin
- Peptides, 47, 50, 54
- Peptones, 47, 54
- Percomorph oil, 420, 441
- Peristalsis, 93
- Pernicious anemia, 280, 282, 284
- Persimmons, 559, 565
- pH of blood plasma, 238; *see also* Hydrogen ion activity
- Pharmacopeia unit of vitamin A value, 413
- Phaseolin, 52, 56
- Phenylalanine, 43, 46, 47, 48, 56, 57, 66, 77
- Phlorizin, 116
- Phosphates as buffers, 239
- Phosphatides, *see* Phospholipids
- Phospholipids (Phospholipins, Phosphatides), 24, 36, 37, 395-396
- Phospholipins, *see* Phospholipids
- Phosphoproteins, 53, 57
- Phosphorus, 50, 220, 222, 223, 226-227, 432-434, 475, 509, 510, 513, 527, 561-565
  - absorption, effect of vitamin D, 433
  - amounts in dietaries, 223, 509, 510, 527
  - amounts needed in nutrition, 251-253, 264-269, 273-277
  - content of infant at birth, 457
  - contents of foods, 271, 562-565
  - distribution in body, 227
  - excretion in fasting, 222
  - in acid-base balance of foods, 239
  - in American dietaries, 223
  - in earth's composition, 220
  - metabolism, 433-434
  - percentage in body, 220
  - radioactive, use of in study of phosphorus metabolism, 268
  - relation to rickets, 432-434
  - requirement, 264-265, 267
  - standard allowances, 491
- Photograph of the Atwater-Rosa-Benedict respiration calorimeter, 143
  - of the Benedict-Roth respiration apparatus, 151
  - of the DuBois respiration calorimeter, 144

- Photosynthesis, 10  
 Phrenosin, 36  
 "Physical regulation," 170-171  
 Physiological fuel values, 128-131  
 Physique as influenced by nutrition, 470-476  
 Pigeons, in determination of thiamin, 358  
 "Pillar concepts," 3-7  
 Pineapple (or juice), 559, 565, 571, 572  
 pH, *see* Hydrogen ion activity  
 pK, *see* Dissociation constant  
 Plans for diets adapted to different economic levels, 528-534  
 Plasma, *see* Blood  
 Plasmon, 553  
 Play of children as a factor in their energy metabolism, 186-189  
     references, 178, 191-192  
 Plums, 337, 559, 565, 567, 572  
 Plus-balance *vs.* optimal retention of calcium, 258-260  
 Polyneuritis, 356, 358  
 Polypeptides, 47, 54  
 Polysaccharides, 18-21  
 Pork, 130, 294, 363, 380, 381, 559, 565, 572  
 Portions, 100-Calorie, 130-131  
 "Post-absorptive state," 150  
 Potassium, 220, 222, 223, 224, 226, 562-565  
     amounts in dietaries, 223  
     contents of foods, 562-565  
     excretion in fasting, 222  
     in acid-base balance of foods, 244  
     in earth's composition, 220  
     percentage in body, 220  
     relation to sodium metabolism, 224  
 Potatoes, 131, 208, 232, 245, 270, 271, 292, 308, 316, 321, 337, 338, 363, 364, 418, 526, 527, 529, 559, 565, 567, 572  
 Potentialities, realization of, as influenced by nutrition, 517-519, 522-525, 534, 536-538, 541-543, 546-550  
 Poultry, *see* Chicken *also* Meats  
 Precision of measurements, 573-577  
 Precision of present knowledge of calcium and phosphorus contents of certain foods, 269-271  
     of calcium and phosphorus requirements, 267  
 Precursors of vitamin A, 402-405  
 Predictions of basal metabolism from height, weight, and age, 152-161  
 Pregnancy, 456-461  
     as influencing nutritional requirement, for calcium, 255  
     for iron, 290  
     for vitamin C, 327, 336  
     energy requirements for, 186  
 Preparation expected of the student of this book, 9  
 Preparation of food, relation to the amount required, 173  
 Primary protein derivatives, 54  
 Principle of natural wholes, 6-7, 492  
 Principles of physical chemistry in the control of internal environment, 504-505, 547  
 Probable error, 573-577  
 Probable errors of mean determinations of calcium, phosphorus, and protein requirements, 269-271  
 Problem of the best use of food, 506-535  
 Problem of optimal nutrition, 8, 253, 326, 331, 335-337, 361-362, 377-379, 421-422, 475-478, 490-491, 504-506, 516-519  
 Production of fat from carbohydrate, 109-111  
 Prolamin of rye, 57  
 Prolamins (alcohol-soluble proteins), 53, 57  
 Proline, 44, 45, 46, 48, 56, 57, 77  
 Properties of enzymes, 81-91  
     of vitamins, *see* under each  
 Proportions of nitrogenous end products, 117-119  
 Prosthetic group, 77  
 Protamins, 53  
 Proteans, 54  
 Proteases, 81, 82  
 "Protective foods," 515-519, 523-534  
 Protein(s), 42-80, 81, 86-90, 94, 95, 100, 101, 193-213, 227, 231-233, 473-474, 490, 509, 510, 513, 527, 555-560  
     absorption and distribution of digestion products, 114  
     adjustment to increased intake, 195  
     to lower intake, 195  
     allowance, 205  
     amino-acid make-up of, 43-50, 55-58

- Protein(s), and their amino acids, as  
 precursors of catalysts, 71-77, 86-90  
 analogy to carbohydrate, 47-48  
 as amphoteric, 51, 52  
 as colloids, 51, 52  
 as factor in growth, 473-474  
 average elementary composition of,  
 138  
 carbohydrate radicle in, 45  
 chemical properties of, 42-61  
 chemistry of, 42-61, 193-202  
 classification, 52-54  
 coagulated, 54  
 colloidal nature of, 50-52  
 comparison of isolated proteins as  
 food, 63  
 "complete," 68  
 conjugated, 53-54  
 contents of foods (quantitative),  
 555-560  
 deficiency conditions not attribut-  
 able to fault of the food, 213  
 derived, 54  
 effects of different levels of intake,  
 205-206  
 efficiencies in nutrition, 206-209  
 energy value of, 126-129, 130, 131  
 equilibrium, 194-196  
   definition, 194  
 fuel value of, 127-128  
 general properties, 42-58  
 grouping of, as to nutritional com-  
 pleteness, 68-69  
 hydrolysis of, 43-50, 55-58  
 "incomplete," 68  
 intermediary metabolism, 114-119,  
 200-202  
 isoelectric point, 52  
 isomers of, 50  
 linkage of amino acids in, 47  
 metabolism of, 114-119, 193-202  
   adjustment to lowered or in-  
   creased intake, 194-196  
   as influenced by body fat, 193  
   by replacement of carbohy-  
   drates by fat, 197-202  
   by withdrawal of carbohy-  
   drate, 197-202  
   in fasting, 193-194  
   in relation to muscular work,  
   209-211  
 molecular weights of, 50-51  
 nitrogen to sulfur ratio, 231-232
- Protein(s), nomenclature, 52-54  
 nutritional chemistry of, 62-80, 193-  
 203  
 occurrence, 42, 52-54, 56-57, 70-71  
 of blood plasma as buffers, 238  
 origin of name, 42  
 "partially complete," 68  
 percentages of amino acids from  
   hydrolysis of, 48-49, 55-58  
 physical properties, 51-54, 238-239  
 plasma, as buffer, 238-239  
 probable molecular weight of, 50-  
 51  
 protecting power, 197  
 quantitative aspects of metabo-  
 lism, 193-218  
 references, 58-61, 77-80, 213-218  
 relation of chemical constitution to  
   food value, 62-71, 77-80  
 relative efficiency for support of  
 growth, 69-71  
 requirement, 203-213  
   factors determining, 205-213  
   for adult maintenance, 203-205  
   for aged, 213  
   for children, 211-213, 473-474  
   for growth, 473-474  
   for men and women compared,  
   204  
   influence of choice of food, 206-  
   209  
   influence of kind of protein, 206-  
   209  
   influence of muscular exercise,  
   209-211  
   in relation to age and growth,  
   211-213  
   *versus* protein standard, 205  
 secondary derivatives, 54  
 simple, 52-53, 56-57  
 solubilities, 52-54  
 sparing, 196-202  
 specific dynamic action, 172-173  
 standard, 205  
 storage in body, 196, 202-203  
 storage of, during pregnancy, 457-  
 458  
 stunting, 473-474  
 supplementary relations of, 69-71,  
 206-209  
 terminology, 52-54  
 ultimate composition of, 50  
 utilization in the tissues, 115-117

- Proteoses, 47, 54  
 Prothrombin, 450  
 Protoplasm, 36, 42, 157, 169  
 Protoplasmic activity, *see* Metabolism  
 Provitamin(s) A, *see* Carotenes, Cryptoxanthin  
 Prunes, 292, 559, 565, 567, 572  
 Psychic secretion in digestive juices, 91, 94  
 Ptyalin, 17, 82, 86  
 Pumpkin(s), 559, 565, 572  
 Purins, 117, 118  
 Pylorus, 92-95  
 Pyranose, 13  
 Pyridoxine, 392, 397-398  
 Pyruvic acid, 107, 110, 116, 200, 360  
 Pyruvic aldehyde, 107; *see* Methylglyoxal  
  
 Quantities in foods and required in nutrition, *see* each nutrient  
 Question whether better nutrition is an economic or an educational problem, 514  
 Quinic acid, 246  
 Quotient, respiratory, 108-110  
  
 Race, as possible factor in basal metabolism, 167-168  
 Rachitic, *see* Rickets, Vitamin D  
 Rachitic rosary, 433  
 Radiation of heat in regulation of body temperature, 170  
 Radishes, 245, 316, 337, 559, 565, 572  
 Raisins, 131, 559, 565, 572  
 Rapeseed oil, 29, 34  
 Raspberries, 559, 565, 572  
 Raspberry juice, 565  
 Rate of growth and length of life, 544-545  
 Rate of oxidation in the body, *see* Catalysis, Energy, Oxidation, Metabolism,  
 Rate of passage of food through the digestive tract, 91-98  
 Ratio, ketogenic-antiketogenic, 113  
 Ratios of calcium to phosphorus, 265-266  
 "Reaction," *see* Hydrogen ion activity  
 Readjustments of expenditure, 523-525, 532-533  
 Reality of findings, 573-577  
  
 Recognition of the far-reaching influence of nutrition, 536-538, 541-546  
 Recommendations for budgeting of expenditure for food, 512, 513  
 Recommendations to guide the choice of foods, 365  
 Redistribution of anions, 240  
 Redox, *see* Oxidation-reduction  
 Redox (oxidation-reduction) systems, 278, 385  
 Red snapper, 316  
 Reductase(s), 81  
 Reduction of body weight, 189-190  
 References, *see* lists at the ends of the respective chapters  
 "Refinement" of foods may greatly lower their nutritive values, 362, 365-366  
 Regeneration, *see* Blood  
 Regeneration of hemoglobin, 280-287  
 Regulation of body temperature, 169-171  
 Relation between expenditures for food and for other things, 523, 524  
 Relation, quantitative, between work performed and total energy metabolism, 180-184  
 Relations between minimal normal and optimal intakes are not the same for all nutrients, 490-491  
 Relationships of vitamin A and its precursors, 402-405  
 Rennin, 81, 82  
 "Repair processes" in protein metabolism, 207  
 Reproduction, 229, 447-448, 456-461 as test of nutritive value of diet, 460-461  
 Requirement, *see* discussion of each nutrient  
 Requirements, calcium, 253-269  
     nutritional, for energy, 134-149  
     as influenced by lactation, 465-466  
     phosphorus, 264-267  
     total energy, 180-192  
 Reserve, alkaline, 239  
 Resistance to disease and premature aging, 326, 330, 331, 334, 374-379, 408, 409, 422  
 Resorption, 97

- Respiration apparatus, 134-137, 140-145, 151
- Respiration, as a factor in acid-base balance, 240-241
- Respiration calorimeter, 140-145
- Respiration experiments to measure energy metabolism, 132, 134-137
- Respiratory-enzyme, 373
- Respiratory metabolism, 134-137, 140-145, 151; *see also* Energy
- Respiratory quotient, 108-110
- Rest and work days compared, 182
- Rest in bed, energy metabolism during, 147
- Results with typical individuals studied in the respiration calorimeter, 146
- Retardation, *see* Growth
- Retention, of calcium in growth, 255-264
- Reticulocytes, 286
- Reversibility of enzyme action, 85
- Rhubarb, 559, 565, 572
- Riboflavin, 373-384, 394, 491, 527, 568-572  
chemical name and structure, 373  
constitution and forms of occurrence, 373-374  
contents of foods, 380-381, 568-572  
distribution in nature, 373-374, 378, 380-381  
formation in nature, 380  
forms of occurrence, 373-374  
in relation to other factors in tissue respiratory metabolism, 378-379  
molecular structure, 373  
quantitative determination, 379-380  
relation to the eye, 375  
relation to the skin, 375  
requirements, 381-382  
"saturation," 382  
significance for nutritional well-being, 374-378  
structural formula, 373  
summary of relations to health, 376-377  
value of the former "unit of vitamin G," 379
- Rice, 99, 244, 350, 363, 559, 565, 567, 572  
polished, 292
- Rickets, 429-440  
definitions of, 432-433  
hypothesis of a local factor in, 434  
low-calcium, 433  
low-calcium and phosphorus, 433  
low-phosphorus, 432-433  
mineral content of blood in, 432-434  
nature of, 432-433  
osteoporosis, 433  
prevention of, 432-434  
relation to growth, 434-435  
relation to mineral content of diet, 432-433  
relation to ultraviolet irradiation, 431-432  
relation to vitamin D, 429-436  
second type of, 433  
tetany in, 433  
"true," 433  
types of, 432-433  
"Rickets-like condition," 433
- Rocks as source of iodine, 307
- Roentgen rays, 92
- Rose-MacLeod chart of basal metabolism, of boys, 163  
of girls, 164
- Roughage, 20
- R.Q., *see* Respiratory quotient
- Rubner factors for fuel value of food, 129
- Rules for simple statistical interpretation of data, 574-576
- Running, 185
- Rutabagas, 245
- Rye, 559, 565, 567
- Saccharose, *see* Sucrose
- Salad dressing, 559
- Salad oil, 558
- Salicylic acid, 553
- Saliva, 91-95
- Salivary amylase, 82, 86
- Salivary digestion, 91
- Salmon, 130, 131, 316, 388, 559, 565, 572
- Salt (table), 223, 224  
as vehicle for the iodine needed in nutrition, 307-314  
iodized, 307-308, 313-315  
*See also* Sodium chloride
- Saponification, 26
- Sardines, 559, 572



- Sauerkraut, 572  
 Sausage, 559  
 Sawing, 185  
 Scallops, 57, 559  
 School-boys, development of, as influenced by food, 477-478, 537  
 Science, division into sciences, 1  
 Scleroproteins, *see* Albuminoids, Collagen, Gelatin  
 Scurvy, 321-322, 327-329, 331-333  
     graded experimental, 328-329  
     history of, 321-322  
     references, 342-347  
     score, 329  
     symptoms of, 325-329  
 Sea as source of iodine, 307, 310  
 Search for the anti-neuritic substance, 351-353  
 Season as factor in growth, 479  
 Season in relation to energy metabolism, 168, 169  
 Seaweeds, 314  
 Secondary protein derivatives, 54  
 Secretin, 96  
 Secretion of gastric juice, 93-96  
 Secretions, internal, as influencing energy metabolism, 162-163  
 Section, horizontal, of the original Atwater-Rosa-Benedict respiration calorimeter, 142  
     vertical, of bed calorimeter, 145  
 Seeds, 339, 363, 380, 381, 418; *see also* name of each  
 Segmentation of food mass in the intestine, 95  
 Serine, 43, 46, 47, 48, 56, 57  
 Serum albumin, 213  
 Serum globulin, 50, 52, 232  
 Serum, normal blood, calcifying power of, 432  
 Sewing, 185  
 Sex, influence of, on basal metabolism, 166  
 Shad roe, 559, 572  
 Shape of body as a factor in basal metabolism, 152-160  
 "Shares," 494  
 Shellfish, 57  
 Shift, chloride, 240  
 Shivering as a factor in the regulation of body temperature, 171  
 Shredded wheat, 560  
 Shrimp, 57, 316, 559, 565, 572  
 Significance, statistical, of findings, 267, 269-271, 337, 573-577  
 Silicon, 220, 227-228  
 Simple deamination, 116  
 Simple lipids, 24  
 Simple proteins, 52-53, 56-57  
 Simple triglycerides, 30-31  
 Single-feeding method for vitamin A values, 415-416  
 Sirup, 559, 565  
 Sitting, 185  
 "Six pillar concepts" upon and around which the chemistry of nutrition is being built, 3-7  
 Size as affecting energy metabolism, 152-161  
 Skeleton, 260-262  
 Skin, formation of vitamin D in, 431  
 Sleep, as affecting metabolism, 169  
 Soaps, 26  
 Sodium, 220, 221, 222, 223, 225-226, 562-565  
     amount in dietaries, 223, 226  
     contents of foods, 562-565  
     distribution in body, 224  
     excretion in fasting, 221-222  
     in earth's composition, 220  
     percentage in body, 220  
     relation to potassium metabolism, 224  
 Sodium chloride, 223-226  
     deprivation of, 224, 225  
 Soluble starch, 19  
 Solvent power, 221  
 Sørensen terminology, 83  
 Soybean(s), 56, 68, 559, 565  
     oil, 35  
 Specific dynamic action of the food-stuffs, 170, 171-173  
 Spermaceti wax, 28  
 Sphingomyelin, 24, 25, 36, 37  
 Sphingosine, 37  
 Spinach, 272, 292, 363, 380, 559, 565, 567, 572  
 Spleen, 294  
 Spray, sea, as source of iodine, 307  
 Sprouting of seeds to develop vitamin C, 339  
 Squash, 559, 565, 572  
 Stability of enzymes, 85, 88, 89  
 Standard deviation, 574  
 Standards, dietary, 489-503

- Standards, dietary, discussion of different types, 492-494
  - for children, 500-501
  - limitations of, 489-491, 496
- Standards for normal basal metabolism, 157-169
  - of Aub and DuBois, 157, 158
  - of Dreyer, 159
  - of Harris and Benedict, 158
- Standing, 185
- Starch, 18-19, 91-92, 95, 127, 245, 553
- Starch sugar, *see* Glucose
- Statistical methods, 480, 573-577
- Statistical treatment of data, 573-577
- Stearic acid, 26, 28, 200
- Stearin (glyceryl tristearate), 26, 200
- Steps, chemical, in the formation of fat from carbohydrate, 110-111
  - in the development of the present-day chemistry of nutrition, 3-7
- Stereochemistry of amino acids, 46
- Sterols, 24, 36, 37, 429; *see also* Vitamin D
- Stomach, 92-95
- Storage, of carbohydrate in the body, 109
  - of fat in the body, 113
  - of nutrients as a factor in growth, 478
  - of vitamin A, 410-412
  - of vitamin D, 436
- Stores of vitamin A in the body, 410-412
- Stratification of food in the stomach, 92-93
- Strawberries, 337, 338, 560, 565, 567, 572
- Stroma, 281, 286
- Structure, *see* Formulas
- Studies of food consumption, 513-515
- Stunting, *see* Growth
- Substrate, 82
- Sucrase, 16, 81, 83, 84, 86
- Sucrose, 14-16, 127, 553; *see also* Sugar
- Sugar(s) (sweets), 245, 508, 510, 512, 526, 527, 529, 560, 572; *see also* name of each sugar
- Suggestions for budgeting of expenditures for food, 512, 513, 523-532
- Sulfate, 231, 242
- Sulfhydryl, 72, 73
- Sulfolipids, 24
- Sulfur, 220, 221, 222, 223, 230-233, 562-565
  - amount in proteins, 232
  - contents of foods, 562-565
  - excretion, 221, 222, 231
  - in acid-base balance of foods, 232-233, 238, 241-242
  - in earth's composition, 220
  - percentages of, in amino acids, 46
  - in body, 220
  - in proteins, 49, 50
  - ratio of, to nitrogen in proteins, 231-232
  - to protein in foods, 232-233
- Sulfuric acid, excretion of, 232-233, 242
- Summary of digestive enzymes, 86
- Summary of evidence on energy requirement as studied by different methods, 147
- Summary of fate of the foodstuffs, 123
- Supplementary relations between proteins, 69-71
- Surface in relation to basal metabolism, 152-159
  - relation of to body weight, 153-156
- Surface of body estimated from height and weight, 154-156
- Survivorship as influenced by nutrition, 542-543
- Susceptibility, *see* Incidence of disease
- Sweating as a factor in regulation of body temperature, 170
- Sweetpotatoes, 245, 418, 526, 527, 529, 560, 565, 567, 572
- Sweets, 339, 364, 418; *see also* Sugar(s)
- Swimming, 185
- Symptothion, *see* Thioneine
- Symptoms of vitamin A deficiency, 406-408
- Synthesis of fatty acids from carbohydrates, hypotheses of Leathes and of Smedley, 110-111
- "Syntonin," 54
- Table(s) of, amino-acid composition of proteins, 48, 56, 57
  - Armsby's comparison of energy computed and found, 144
  - basal metabolism of adults, 158
  - of children, 165
  - calcium contents of foods, 270, 562-565

- Table(s) of, carbohydrate content of foods, 555-560  
 chlorine contents of foods, 562-565  
 coefficient of digestibility of foods, 101  
 copper contents of foods, 566-567  
 data on influence of food upon length of life, 518, 519  
 data on mechanical efficiency of man, 182  
 digestive enzymes, 86  
 dissociation constants and normal excretion of organic acids, 243  
 distribution of calories in typical foods, 131  
 distribution of cost and nutrients in American dietaries, 510, 527  
 distribution of energy values of foods between protein, fat, and carbohydrate, 131  
 distribution (Van Slyke) of nitrogen in proteins, 58  
 distribution standards, 500, 501  
 elementary composition of amino acids, 46  
 elementary composition of body fat and protein, 138  
 elementary composition of proteins, 50  
 elementary composition of the body and its environment, 220  
 energy expenditures per hour, 185  
 energy metabolism during sleep, 152  
 energy values of foods, 131, 555-560  
 energy values of oxygen and carbon dioxide at different respiratory quotients, 136  
 factors for use with the oxy-calorimeter, 553-554  
 fat contents of foods, 555-560  
 food allowances for children, 500, 501  
 food consumption in different economic conditions, 515, 529  
 foods in which acid-forming elements predominate, 244  
   in which base-forming elements predominate, 245  
 heats of combustion as related to elementary composition, 127  
 100-Calorie portions of foods, 131, 555-560  
 indispensable amino acids, 66
- Table(s) of, iodine contents of foods, 316  
 iron contents of foods, 292, 562-565  
 low outputs of nitrogen in different species, 202, 203  
 magnesium contents of foods, 562-565  
 manganese contents of foods, 566-567  
 mineral elements in foods, 561-567  
 nitrogen balances, 195, 198, 199  
 nitrogen distribution in urine as influenced by level of protein intake, 118, 203  
 phosphorus contents of foods, 271, 562-565  
 potassium contents of foods, 562-565  
 protein contents of food, 555-560  
 proximate composition and energy values of foods, 555-560  
 riboflavin contents of foods, 380, 568-572  
 sodium contents of foods, 562-565  
 sulfur contents of foods, 232, 562-565  
 surface area of body, 154  
 thiamin contents of foods, 363, 568-572  
 urinary excretion of different elements during a 31-day fast, 222  
 vitamin A values of foods, 418, 568-572  
 vitamin C contents of foods, 337, 568-572  
 vitamin values of foods, 568-572  
 Tangerines, 572  
 Tapioca, 560, 565  
 Tartaric acid, 247  
 Taurocholic acid, 96  
 Teeth, 221, 228  
 Temperature, body, regulation of, 169-171  
 Temperature as influencing stability of vitamin C, 341  
 Temperature of environment as influencing food consumption and growth, 479  
 Tension (tone) of muscles as a factor in energy metabolism, 150, 151, 152  
 Terminology, of enzymes, 82  
   of fats and lipids, 24

- Terminology, of proteins, 52-54
- Testing for vitamin A values of foods, 412-416
- Tetany, in low-calcium rickets, 433
- Tetradecenoic acid, 30
- "The body's first line of defense," 409
- Thiamin, 348-372, 527, 568-572
  - bacterial synthesis in rumen of cow, 464
  - chemical nature, 351-352
  - content of foods, 362-367, 568-572
    - as affected by cookery, 362
    - effect of refining, 362
  - deficiency, 348-351, 356
  - determination of, bradycardia method, 358
  - in vitro, 359
  - microorganism method, 359
  - prevention or cure of polyneuritis in birds, 358
  - rat growth method, 356-357
  - U. S. Pharmacopeia method, 358
- distribution in foods, 362
- effect of shortage, 356-357
- function in the intermediary metabolism of carbohydrates, 354
- functions in nutrition, 353-355
- in carbohydrate metabolism, 354
- in foods, 362-367, 568-572
- in metabolism of carbohydrate, 354
  - of fat, 360
  - of pyruvic acid, 354
- in normal nutrition, 353-355
- in promotion of appetite, 353, 354
- in relation to other factors in tissue respiratory metabolism, 378-379
- influence of level of feeding, 355-357
- influence on appetite, 353, 354, 355
- influence on growth, 353, 354, 355
- influence on motility of the digestive tract, 354
- influence on the metabolism of pyruvic acid, 354
- international unit, 359
- isolation and chemical identification, 351-352
- method for demonstration and measurement, 355-359
- molecular structure, 352
- needed in proportion to non-fat calories metabolized, 359, 360
- nutritional functions of, 353-355
- Thiamin, occurrence, 362-367, 568-572
  - origin of the name, 352
  - problem of adequacy of ordinary intake, 365-367
  - problem of optimal intake, 361-362
  - quantitative determination, 355-359
  - relation to appetite, 361
    - to motility of the digestive tract, 354
    - to polyneuritis, 356-358
  - requirement, 359-361
    - determination of, 360
    - pregnant and lactating women, 361
  - requirements and standards, 359-362
  - saturation of body with, 361-362
  - "saturation studies," 361
  - structure, 352
  - tonic effect of, upon the digestive tract, 354
  - units, 359
  - values of foods, 362-367, 568-572
    - methods of measuring, 355-359
- Thiasine, *see* Thioneine
- Thiazole, 352
- Thioamino acid, *see* Cystine, Methionine
- Thioneine, 230-231
- Threonine, 43, 46, 66
- Thrombase, 81
- Thrombin, 81
- Thymo-nucleic acid, 53
- Thymus histone, 53
- Thyroglobulin, 309, 311
- Thyroid, 307-315
  - iodine content of, 311
- Thyroxine, 73-74, 202, 309-312
  - structure of, 73
- Time, relation of, to destruction of vitamin C, 341
- Tissue-respiration metabolism, 378-379
- Tissues, acid-base balance of, 247-248
- Tocopherol(s), 447-448, 450-452
- Tomatoes (or juice), 131, 245, 292, 316, 325, 328, 337, 338, 340, 341, 363, 380, 388, 418, 515, 526, 527, 529, 531, 560, 565, 567, 572
- Tone of the muscles as a factor in energy metabolism, 151, 152

- Tongue, 560  
 Tonus of muscles as a factor in basal metabolism, 150, 151  
 Trabeculae, 253-254  
 Traditional food habits, significance of, 536  
 Treatment, statistical, of the data of nutrition investigations, 573-577  
 Triglycerides, 30-31; *see also* Fats  
 Tripeptides, 47, 54  
 Tritico-nucleic acid, 53  
 Trypsin, 82, 83-84, 86, 88, 90  
 Tryptophane, 45, 46, 47, 48, 49, 56, 57, 63-64, 66, 72, 86  
 Tuna (tunny), 560  
 Turkey, 560, 565  
 Turnip(s), 245, 270, 271, 292, 337, 338, 364, 380, 418, 560, 565, 567, 572  
 Turnip greens, 364, 388, 560, 565, 572  
 Types of dietary standards, 492  
 Types of food, 507-513, 515-516, 526-531  
 Typewriting, 185  
 Tyrosine, 43, 46, 47, 48, 56, 57, 74, 75, 77  
  
 Ultimate composition, of amino acids, 46  
     of proteins, 49, 50  
 Ultraviolet irradiation, in cure or prevention of rickets, 432-434  
     in formation of vitamin D, 431, 432  
     in prevention of rickets, 434  
 Ultraviolet light, activating wavelengths, 431-432  
 Under-consumption of protective foods, 523-525  
 Urea, 117, 118, 119, 127, 128, 202, 203  
 Urease, 88  
 Uric acid, 117, 118, 119, 128, 243, 533  
 Urine, ammonia in, 118  
     as a factor in acid-base balance, 241-242, 246  
     buffers in, 241-242  
     hydrogen ion activity of, 241-242  
     pH of, 241-242  
 Use of food, problem of the best, 506-535  
 Uses of the organic foodstuffs, summary, 123  
 U. S. Pharmacopeia, method of determining thiamin, 358  
  
 Utilization, of energy value of food, summary, 123  
     of protein, 115-117  
     of the calcium of different foods, 272-273  
  
 Validity, of averages for calcium and phosphorus contents of foods, 270-272,  
     of findings, 573-577  
 Valine, 43, 46, 48, 49, 56, 57, 66  
 Value(s) of foods as sources of energy, 123-131, 555-560  
 Value as distinguished from content (vitamin A), 402  
 Value of home growing of food for health, 533-534  
 Value of the individual to society as influenced by nutrition, 522, 536, 542-550; *see also* Ability  
 Van Slyke distribution of amino acids in proteins, 55, 58  
 Van Slyke method, 55, 58  
 Variation, coefficient of, 574  
 Variations, natural, affected by many causes, 573-577  
 Veal, 294, 560, 565  
 Vegetable(s), 21, 98, 101, 336, 363, 364, 365, 366, 376, 380, 388, 500, 501, 508, 510, 512, 513, 515, 516, 526, 527, 529, 531, 534  
 Vegetarianism and basal metabolism, 168  
 Vicilin, 56  
 Vignin, 52, 56  
 Vinegar, 565  
 Viosterol, 431; *see also* Irradiated ergosterol  
 Vision, 405-406  
 Vitality increased by liberal intake of calcium, 260, 539-540  
     of riboflavin, 377, 540  
     of vitamin A, 422, 540-541, 543  
 Vitamin A, 402-427, 478, 527, 568-572  
     chemical nature, 402-405  
     clinical evidences of deficiency, 406-408  
     clinical observations, 407  
     contents of foods, 568-572  
     deficiency, 407-410  
     demonstration and measurement of values, 412-416  
     desirable level of intake, 491

- Vitamin A, discovery, 405  
 distinction between minimal and optimal standards, 421-422  
 distribution in body, 410-412  
   in nature, 402, 416-420  
 experimental deficiency, 407-410  
 formation in nature, 402-405, 416-417  
 frequency of shortage, 406, 407  
 functions in nutrition, 405-412, 421-422  
 in American dietaries, 527  
 in grasses, 419  
 influence upon growth, 405, 412-416  
 methods for demonstration and measurement, 412-416  
 nutritional functions, 405-412, 421-422  
 origin in grasses, 416, 419-420  
   in sea foods, 420  
 precursors of, 402-405  
 problem of optimal intake, 491  
 quantitative measurement of, 412-416  
 quantitative requirements of human nutrition, 421-422  
 relation to carotenes, 402-405  
   to the eyes, 405-407, 421  
   to growth, 405, 412-416  
   to health, 405-410  
   to xerophthalmia, 407  
 shortage as cause of metaplasia, 407  
 significance in nutrition, 402, 405-422  
 storage in the body, 410-412  
 structure, 403  
 survival value, 405  
 unit of value, 413  
 value, determination of, 412-416  
   regular feeding method, 413-416  
   single-feeding method, 415-416  
 value, international and U.S.P. unit, 413  
 value (distinguished from content), 402  
   values of foods, 416-420, 568-572
- Vitamins A<sub>1</sub> and A<sub>2</sub>, 402
- Vitamin, antineuritic, *see* Thiamin  
 antirachitic, *see* Vitamin D  
 antiscorbutic, *see* Vitamin C
- Vitamin B<sub>1</sub>, *see* Thiamin
- Vitamin B<sub>2</sub>, 391
- Vitamin B<sub>4</sub>, 391
- Vitamin B<sub>5</sub>, 391
- Vitamin B<sub>6</sub>, 392, 397-398
- Vitamin C, 321-347, 491, 527, 568-572  
 action in prevention of scurvy, 321-322  
   in prevention of systemic pyorrhea, 327  
   in promotion of positive health, 326, 329-337  
 blood plasma values, 326, 331-337  
 bodily stores, 330-337  
 chemical nature, 323-325  
 chemical relationship to monosaccharides, 323  
 clinical observations, 329-335  
 concentration-levels in the blood, 326  
   in the body, 326, 329-337  
 consequences of lack, 325-329; *see also* Scurvy  
 conservation in foods, 324-325  
 contents of foods, 568-572  
 deficiency, 325-329  
 demonstration of graded degrees of deficiency, 328-329  
 destruction of, 323, 324, 325  
   in the body under the influence of infections, 330  
 diminution in heated food, 341  
   in stored food, 339  
 discovery and chemical identification, 321-324  
 distribution in foods, 337-340, 568-572  
   in tissues, 334-335  
 effect on body's resistance to bacterial toxins, 330  
 effect upon intercellular cement substance likened to reinforced concrete, 327  
 experimental deficiency, 327-329  
 foods as sources of, 337-341  
 formation in sprouting seeds, 339  
 identification, 322  
   in body tissues, 334-335  
 increased metabolism in athletics, 336  
 individual differences in metabolism and need, 330-333  
 influence of liberal intakes upon complement-value of blood, 326

- Vitamin C, influence of pH, 340, 341  
     of temperature, 341  
     influence of shortage, upon bones, 325, 326, 328-329  
         upon hemorrhages, 325, 327-329  
         upon intercellular cement substance, 325-326  
     influence of the level of intake upon concentration-level in the body, 331-332  
     international unit, 325  
     losses of, 323-324, 325, 340-341  
         in cooking, 340-341  
     molecular structure, 322  
     more than merely an antiscorbutic, 326  
     numerical expression of contents in foods, 325  
     nutritional functions, 325-337  
     oxidation of, 323, 324, 325  
     oxidation-reduction potential, 323, 324, 325  
     percentage recovery of test dose as indication of body condition, 330  
     postulation as a definite substance, 322  
     problem of optimal intake, 326, 329-337  
     problem of possible synthesis in the digestive tract of cow, 464  
     quantitative determination, 324-325  
     rate at which used-up in the body, 330-337  
     references, 342-347  
     relation, to diphtheria toxin, 326, 330  
         to immunological complement, 326  
         to infections, 326-327, 330-331  
         to intercellular cement substance, 325-326  
         to nutrition and health, 325-337  
         to teeth, 327-328  
     requirement as influenced by various conditions, 326-327, 330-337  
     "saturation," 336  
     sources in the food supplies of the Eskimos, 331  
     stability as influenced by pH, 325, 340-341  
     by temperature, 341
- Vitamin C, structural formula, 323  
     structure, 323  
     suggested standards, 335-336  
     susceptibility to oxidation, 324-325  
     symptoms developed by graded experimental deficiency, 328-329  
     symptoms of shortage as influenced by bodily constitution, 326  
     synthesis, 322, 323  
     synthetic, 323  
     units, 325  
     values of foods, 337-341, 568-572  
         methods of measuring, 324, 325, 328-329
- Vitamins D, 429-446  
     discovery, 429  
     formation by irradiation, 431, 432  
     human requirement of, 438-440  
     in butter, 440, 441  
     in egg yolk, 440, 441  
     in fish liver oils, 441  
     in irradiated substances, 431-432  
     in milk (fat), 440, 441  
     in natural foods, 440-441  
     international standard, 437  
     isolation of natural, 430  
     line test in the assay of, 436-438  
     measurement and expression of values (potencies), 436-438  
     nutritional functions, 434-436  
     question of need for adult maintenance, 440  
     relation to calcium and phosphorus metabolism, 433-434  
         to growth, 434, 435-436  
         to teeth and bones, 432-436  
     relative efficacy in infant, chick, and rat, 431-432  
     requirement in childhood, 439-440  
         in infancy, 439  
         in pregnancy and lactation, 440  
     sources, 440-442  
     stability, 441  
     storage in the body, 436  
     structure, 429-431  
     transfer from mother to young, 436  
     units, 437
- Vitamin D<sub>2</sub>, 430, 431  
     Vitamin D<sub>3</sub>, 430, 431  
     Vitamin D milk, 441  
     Vitamin E, 447-448, 450-452; *see also* Tocopherol(s)

- "Vitamin F," 448  
 Vitamin G, *see* Riboflavin  
 "Vitamin H," 393  
 Vitamin K, 440-450, 453-455  
 Vitamin K<sub>1</sub> and K<sub>2</sub>, 449  
 Vitamin M, 393, 398  
 Vitamin P, 391, 393, 398  
 Vitamin(e), origin of the word, 351  
 Vitamin(s), concept, 322  
 Vitamins, general, 4-5  
     nomenclature, 322  
     *See also* Ascorbic acid, Nicotinic acid, Thiamin, Riboflavin  
 Vividiffusion (reference), 119
- Walking, as influencing energy metabolism, 181-182, 185  
     energy requirement (or energy cost) of, 137  
 Walnuts, 560, 565, 572  
 Water, as a factor in body weight, 133  
     as source of iodine in nutrition, 307-308, 310, 313-315  
     supplies, and prevalence of goiter, 308  
 Watercress, 560, 565, 572  
 Watermelon, 245, 337, 364, 560, 565, 572  
 Water-soluble B, *see* Thiamin  
 Water-soluble C, *see* Vitamin C  
 Water-soluble vitamins, 321-401  
 Waxes, 24  
 Weight as indication of adequacy of total food (energy) intake, 497  
 Weight, control of, 189-190, 497  
     fluctuations of, 133  
     relative gain by children, 211  
 Weight-height-age relationships, 152-161, 163-166, 494-500  
 Weight-surface relationships, 152-161  
 Welfare as influenced by nutrition, 522-550  
 Wheat, 52, 53, 56, 57, 68, 232, 244, 270, 271, 285, 292, 295-296, 316, 357, 363, 380, 560, 565, 567, 572  
     shredded, 131, 560  
 Wheat-germ, 380, 388, 572  
 Wheat-plant as sole source of ration, 459-460  
 Whole-grain products, 365-366, 367, 501  
 Wholes, 6-7, 492  
 Wine, 565  
 Wool wax, 28  
 Work, after-effect of, upon energy metabolism, 152  
     mechanical efficiency of, 181-184  
     muscular, 123  
         as affecting the immediate hourly metabolism of energy, 180-185  
         as affecting total food (energy) requirement, 180-189  
         references, 191-192  
         in regulation of body temperature, 170
- Xerophthalmia, 407; *see also* Vitamin A  
 X-rays, 92
- Years of dependence as influenced by nutrition, 377  
 Yeast, 285, 572  
     high-thiamin, 366  
 Yolk, egg, 292; *see also* Egg yolk  
 Young, nutrition of, *see* Children, Dietary standards, Growth  
 Youth, *see* Children, Growth, Health
- Zein, 48, 49, 50, 53, 57, 63, 64, 68, 69-71, 232, 473  
 Zinc, 220, 230  
 Zuntz-Schumburg factors, 136  
 Zwitter-ion theory, 51  
 Zymase, 82  
 Zymogen, 82; *see also* Enzymes.









